SHORT COMMUNICATION

Moderate antiproliferative effect of the antifolate CB3717 in the BN myeloid leukaemia model

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The novel antifolate CB3717 (N\textsuperscript{16}-propargyl-5,8-dideazafolic acid) has proved to be a potent inhibitor of thymidylate synthetase (TS) (Jones et al., 1981), the enzyme providing the \textit{de novo} synthesis of thymidylate (dTMP) from uridylylate (dUMP). Distinct antiproliferative activity of the drug has been observed in L1210 ascites tumour (Jones et al., 1981) and in human hepatoma xenografts (Curtin et al., 1986). A phase I trial has revealed that side effects in man consists mainly of hepatotoxicity and renal dysfunction, the latter limiting the maximal applicable dose to 600 \text{mg} \text{m}^{-2} (Calvert et al., 1986).

A specific benefit of the drug is the absence of effect on purine synthesis, which possibly reduces toxic side effects. Moreover CB3717 does not need metabolic activation, making it less vulnerable to certain forms of drug resistance.

In this study the chemotherapeutic activity of CB3717 was investigated in the Brown Norway myeloid leukaemia (BNML) and compared with 5-fluorouracil (5-FU). This pyrimidine analogue has proved to be a potent inhibitor of TS, although its effect on cell proliferation is also related to incorporation into DNA and RNA. Moreover the antitumour effects of both drugs were correlated with their \textit{in vitro} inhibition of TS activity.

Properties of the BNML have been described before and this rat leukaemia is considered to be a reliable model for experimental chemotherapy (Hagenbeek et al., 1977). Briefly, rats were injected i.v. with 10\textsuperscript{7} leukaemic cells which, after progressive leukaemic infiltration of bone marrow, liver and spleen lead to death in 20-24 days. Increases of spleen and liver weight have proved to be reliable indicators of leukaemic growth. In the present study antileukaemic effects were evaluated by these parameters 18 days after transfer of leukaemia. Rats were sacrificed by exsanguination and liver and spleen removed and weighed. Liver and kidneys were macroscopically inspected in order to assess possible toxic effects of treatment. Peripheral leucocytes were counted electronically.

CB3717, kindly provided by ICI Pharmaceuticals (Macclesfield, UK), was dissolved in 0.15 M NaHCO\textsubscript{3}, pH 9. Both CB3717 and 5-FU (Hoffmann-La Roche) were administered on day 9, 12 and 16 after leukaemic transfer or on 5 consecutive days, starting on day 9 in the case of the 5 day protocol.

In one experiment a leukaemic cell suspension (5 \times 10\textsuperscript{6} cells ml\textsuperscript{-1}) in Hanks balanced salt solution was prepared from 2 massively infiltrated spleens. One hundred \textmu l aliquots of this suspension were preincubated with various concentrations of CB3717 or 5-FU for 1 h and subjected to the deoxyuridine (dU) suppression test (Matthews and Wickramasinghe, 1986). The incorporation of \textsuperscript{3}H-thymidine into DNA with or without dU (0.3 \textmu M) is measured. After 1 h preincubation with dU reduces \textsuperscript{3}H-thymidine incorporation if its metabolite dUMP can be converted to dTMP through TS; impaired activity of the latter enzyme will reduce this suppressive effect of dU on \textsuperscript{3}H-thymidine incorporation. The test has proved to be a useful tool for the measurement of drug activity directed at \textit{de novo} dTMP synthesis (Bruckner et al., 1975; Kroes et al., 1986).

In the first series of experiments, leukaemic rats were treated with 10-30 mg CB3717 kg\textsuperscript{-1} body wt. as currently used in human phase II trials (Cantwell et al., 1986). Table I shows that no antileukaemic effect of the treatment schedules was apparent and that signs of toxicity were not present. On increasing the doses of CB3717 to 100-125 mg kg\textsuperscript{-1} body wt., levels that exhibit therapeutic activity in the L1210 leukaemia (Jones et al., 1981), severe weight loss and increased water consumption, were observed. Finally death of all treated animals on day 17 or 18 of leukaemia occurred, which is just before the assigned time of evaluation. Autopsy revealed some reduction of spleen and liver weights through treatment but macroscopic visible granulation and discolouring of liver and kidneys, probably due to toxicity, were also noticed. As toxicity of CB3717 is possibly related to its peak plasma levels (Alison et al., 1985; Newell et al., 1982), the route of administration was changed from the bolus i.v. to the i.p. route. Table I shows that with 3 \times 100 mg or 5 \times 75 mg CB3717 i.p. fewer toxic deaths did indeed occur and that antitumour effects were even slightly enhanced. However, inspection of the intraperitoneal cavity showed evident precipitation of the drug. Consequently, further escalation of the dose CB3717 by this route of administration to improve treatment, was useless.

Compared to CB3717, the pyrimidine analogue 5-FU, administered 3 \times 25 mg kg\textsuperscript{-1} body wt., showed a remarkable antileukaemic effect resulting in almost normal values of the studied parameters without any sign of toxicity as previously described (Ermens et al., 1986).

Because CB3717 and 5-FU both interfere primarily with TS activity, their varying inhibition of leukaemic growth may be reflected in a different potency to change the dU suppression value of leukaemic cells \textit{in vitro}. Figure 1 shows that the lowest concentration of 5-FU tested (3.3 \mu M) already strongly increased incorporation of \textsuperscript{3}H thymidine into DNA in the presence of dU. With CB3717 a similar effect could only be achieved with drug concentrations in the millimolar range.

The present study thus shows a remarkable parallelism between the antileukaemic potential of CB3717 and 5-FU \textit{in vivo} and their influence on \textit{in vitro} dTMP synthesis as measured in the dU suppression test.

The reason for the relatively limited \textit{in vivo} benefit of high doses of CB3717 on the BNML is unclear. Other studies have revealed that inhibitory effects of CB3717 on DNA synthesis and cell proliferation develop slowly (Simpson & Harris, 1985) or require higher concentrations (Jackman et al., 1986) compared to 5-FU. It is possible that limited cellular uptake of the antifolate partly accounts for this. Pharmacokinetic studies in mice have shown that i.p. administration of 100 mg CB3717 kg\textsuperscript{-1} body wt. results in peak plasma levels of 1.3 \times 10\textsuperscript{-4} M (Newell et al., 1986). Figure 1 shows that CB3717 affects \textit{de novo} dTMP synthesis after 3 h of \textit{in vitro} incubation in this concentration range. However \textit{in vivo} plasma levels of the drug decline to 10\textsuperscript{-7} M within 24 h of administration (Newell et al., 1986). So the
Table I Therapeutic effects of CB3717 and 5-fluorouracil (5-FU)

| Treatment (mg kg⁻¹) | Number of rats | Number of toxic deaths | Liver weight (g ± s.e.m.) | Spleen weight (g ± s.e.m.) | Peripheral leukocytes (10⁶ ± s.e.m.) |
|---------------------|----------------|------------------------|---------------------------|---------------------------|-------------------------------------|
| None (controls)     | 10             | 0                      | 16.82 ± 0.60              | 4.18 ± 0.13               | 24 ± 2.6                            |
| CB3717 3 x 10 i.v.  | 5              | 0                      | 16.10 ± 0.43              | 3.75 ± 0.21               | 23 ± 2.2                            |
| CB3717 3 x 20 i.v.  | 4              | 0                      | 16.44 ± 0.23              | 3.66 ± 0.17               | 20 ± 1.3                            |
| CB3717 3 x 30 i.v.  | 3              | 0                      | 15.74 ± 1.31              | 3.41 ± 0.40               | 20 ± 1.7                            |
| CB3717 3 x 100 i.v. | 4              | 4                      | 14.77 ± 0.48              | 3.59 ± 0.30               | -                                   |
| CB3717 3 x 125 i.v. | 4              | 4                      | 13.92 ± 0.37              | 3.23 ± 0.19               | 11 ± 1.0                            |
| CB3717 3 x 100 i.p. | 6              | 1                      | 13.57 ± 0.35              | 3.26 ± 0.12               | 13 ± 2.4                            |
| CB3717 5 x 75 i.p.  | 4              | 1                      | 11.12 ± 1.83              | 3.21 ± 0.36               | 13 ± 2.4                            |
| 5-FU 3 x 25 i.p.    | 9              | 0                      | 9.02 ± 0.99               | 0.90 ± 0.19               | 5.7 ± 0.8                           |
| Normal BN-rats      | 16             | -                      | 8.25 ± 0.24               | 0.45 ± 0.02               | 3.9 ± 0.4                           |

Figure 1 H-thymidine incorporation into DNA in the presence of dU as a percentage of values without dU. One hour preincubation with 5-FU: ▲, one hour preincubation with CB3717: ●.

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exposure of the leukaemic cells to drug levels necessary for sustained TS inhibition, is probably limited. Thus the ineffectual action of CB3717 on the rat leukaemia studied apparently results from its retarded effect on the target enzyme TS (relative to 5-FU) in combination with a relatively rapid plasma clearance.

The efficacy of CB3717 on the L1210 leukaemia growing i.p. as reported by Jones et al. (1981) can likewise be related to the i.p. administration of the drug which results in specific exposure of the leukaemic cells to high concentrations of CB3717. The observed formation of polyglutamate derivatives of this antifolate in L1210 cells (Sikora et al., 1986), enhancing its cellular retention and activity (Cheng et al., 1985) may also contribute to the therapeutic success of CB3717 in this mouse leukaemia model. From the present study it can be concluded that further preclinical investigations with CB3717 are necessary before this drug can be submitted to trials in human haematological malignancies. In this context, the recent introduction of the 2-desamino derivative of CB3717, which has proved to cause less renal and hepatic toxicity (Jackman et al., 1987), may contribute to improvement of the therapeutic index of this novel antifolate.
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