AN EXPERIMENTAL APPROACH TO INCREASE SELECTIVE TUMOUR TOXICITY OF METHOTREXATE

M. H. N. Tattersall

Charing Cross Hospital (Fulham)

Fulham Palace Road, London W6 8RF

Thymidine protects mice bearing leukaemia L1210 and P388 from methotrexate toxicity without impairing antitumour activity. When thymidine containing pellets were implanted subcutaneously in L1210 leukaemia bearing mice, the therapeutic dose range of methotrexate was increased three-fold. The toxic effects of methotrexate in mice may be ascribed to a disturbance of de novo thymidylate synthesis but the antitumour activity must be due to a disturbance of some other folate dependent pathway.

Introduction

The combination of high dose methotrexate and folinic acid rescue has been used clinically for several years (1,2). The enhanced therapeutic index of the combination compared to methotrexate alone has been established in some experimental tumour systems (3) and in patients with head and neck tumours and osteogenic sarcoma (4,5). Delay in administration of folinic acid after methotrexate is necessary for optimal tumour effect in experimental animals but the optimal timing of the rescue in man has not been established. The biological basis for the increased therapeutic effect of methotrexate folinic acid is not known but a number of hypotheses have been advanced (6). Recently an alternative approach to methotrexate rescue has been proposed. When given at appropriate time intervals after methotrexate treatment, asparaginase prevents the bone marrow toxicity of methotrexate and the antitumour effects of the combination are not necessarily similarly reduced. L Asparaginase reduces the cytotoxicity of methotrexate by inhibiting protein synthesis thus preventing the entry of cells into the DNA synthetic phase of the cell cycle during which cells are sensitive to methotrexate (7). The value of this combination in the treatment of acute leukaemia has been reported recently (8). We have studied the effect of
thymidine on the toxicity and antitumour activity of methotrexate in leukaemia bearing mice. Our result indicate that thymidine decreases markedly methotrexate toxicity but does not reduce the antitumour effect. Thymidine allows substantially greater doses of methotrexate to be administered to tumour bearing mice and the antitumour activity of methotrexate has been increased.

Materials and Methods

Thymidine was purchased from Nutritional Biochemical Corporation, cholesterol from Aldrich Chemical Co., and methotrexate and folinic acid were obtained from the Division of Cancer Treatment, National Cancer Institute. 250 mg cholesterol pellets and thymidine pellets each containing 60 mg of thymidine and 190 mg cholesterol were pressed using a Wabash hydraulic press. Drugs for injection were dissolved in water and administered intraperitoneally or subcutaneously.

Animal Studies

The toxicity of the drugs in non tumour bearing BDF1 mice was studied by daily weighing and survival of drug treated animals compared to controls. The antitumour activity against rodent ascitic tumours was studied in vivo. The cell lines were carried by weekly serial transplantation. The lines of L1210 and P388 used in the experiments have been maintained for several years in this department. 10⁷ cells of L1210 and 10⁶ cells of P388 were inoculated routinely intraperitoneally, and drug treatment was started 24 hours later. Thymidine and cholesterol pellets were implanted subcutaneously in the nape of the neck, and left in place for 3 days. In some experiments the pellets were changed every three days for a total of ten days.

Results

Table 1 shows the effect of simultaneous administration of methotrexate (MTX) and thymidine (TdR) or folinic acid (CF) on normal mice. The results indicate that TdR will prevent the toxicity of 250 mg/kg MTX but not 300 mg/kg, whereas CF prevents toxicity of MTX at 300 mg/kg. Chart 1 shows the weight changes in these animals, and indicates that CF is superior to TdR in preventing the weight loss caused by MTX treatment. Tables 2 and 3 show the effect of TdR given intraperitoneally (ip) and subcutaneously (sc) on the toxicity and antitumour activity of MTX in leukaemia L1210. The results indicate that the simultaneous administration of TdR both ip and sc with MTX protects animals to a large degree from the toxicity of MTX and does not inhibit the antitumour activity. The results also show that TdR alone does not have any antitumour or toxic effects. Table 4 indicates that TdR prevents the toxicity of MTX in animals bearing P388 leukaemia, without inhibiting antitumour activity. In this experiment MTX at 200 mg/kg as a single dose was toxic in the majority of mice, but TdR completely prevented toxicity.