KINETICS OF METOPRINE, A LIPID-SOLUBLE ANTIFOLATE

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1 Using a dihydrofolate reductase inhibition assay, we have conducted eight pharmacokinetic studies in six patients receiving metoprine.

2 The serum level v time-curve for metoprine equivalents was irregular; first-order elimination was not observed during the study period of 0-120 h. A model-independent analysis was therefore performed, employing the area under the curve during the first 120 h (AUC).

3 At an oral dose of 65 mg/m² without leucovorin, a peak level of 0.6 µg/ml and an AUC of 52 µg ml⁻¹ h produced significant leukopenia and thrombocytopenia.

4 At doses ranging from 100 mg/m² to 175 mg/m² orally, with leucovorin administration 40 mg/m² intravenously at 24 and 96 h, haematologic toxicity was seen in only 1 patient who received 175 mg/m². This patient also had the highest peak serum level (2.8 µg/ml) and AUC (228 µg ml⁻¹ h).

5 One partial response and one minor regression were observed in the studied patients; these two patients had the 175 mg/m² dose, highest peak levels (2.4 and 2.8 µg/ml) and highest AUCs (197 and 228 µg ml⁻¹ h). The other patients had lower peak levels and AUCs and had neither therapeutic response nor hematologic toxicity.

6 The AUC and peak serum levels were linearly related to each other ($P < 0.001$).

7 Total urinary excretion of dihydrofolate reductase inhibitors (as metoprine equivalents) in the first 120 h ranged from 5 to 17% of the administered dose.

Introduction

Metoprine (DDMP) (2,4-diammino-5-(3',4'-dichlorophenyl)-6-methylpyrimidine (Figure 1) is a lipid-soluble inhibitor of dihydrofolate reductase that produces antitumor effects in mice (Burchenal, Goetchius & Stock, 1952; Sirotnak, Dorick & Moccio, 1976), rats (Denlinger, Cavallito & Nichol, 1976), and man (Murphy, Ellison & Karnofsky, 1954). Phase I and early Phase II clinical trials in various centres have shown activity in hypernephroma, epidermoid carcinoma arising in bronchus or head and neck, central nervous system leukaemia, malignant melanoma, and mycosis fungoides

(Murphy *et al.*, 1954; Alberto, Brugarolas, & DeJager, 1979; Currie, Kempin & Young, 1981; Miller, Nichol & Rundles, 1976; Price, Goldie & Hill, 1975; Price & Hill, 1976; Price, Goldie & Hill, 1977). In the murine sarcoma 180 model, improvement in the therapeutic index of the DDMP has been noted with the incorporation of citrovorum-factor rescue into dosage schedules (Price & Hill, 1976); clinical studies at Memorial Hospital were conducted to evaluate this regimen in man. In some clinical trials, DDMP produced erratic and unpredictable toxicity (Currie *et al.*, 1981; Newman, Allen & Griffin, 1979). While leukopenia, skin rashes, headaches, nausea and dyspepsia are seen in some patients, the usual dose-limiting toxicity is thrombocytopenia. The drug has a long and variable duration of action (Stickney, Deangelis & Simmons, 1973) leading to accumulation and side effects related to the serum level (Miller *et al.*, 1976). As part of a clinical evaluation of metoprine, kinetic data were acquired under clinical conditions to provide additional information about the drug's efficacy and haematological toxicity.

Figure 1 Structure of metoprine (DDMP).

Methods

Patient selection

Six patients with histological evidence of advanced malignancy (one with epidermoid carcinoma of lung, two with adenocarcinoma of lung, three with mycosis fungoides) in whom conventional therapy had failed were invited to participate in this study. Each patient had a performance status $\geq 50\%$ (Karnofsky Scale) and an estimated survival of at least 8 weeks. No patient had received other therapy for at least 4 weeks prior to receiving metoprine. All patients had total leukocyte count $\geq 4.0/\text{mm}^3$ and platelet count $\geq 120,000/\text{mm}^3$ as well as a serum bilirubin $\leq 1.5 \text{ mg}/100 \text{ ml}$ and serum creatinine $\leq 2 \text{ mg}/100 \text{ ml}$. Voluntary and informed consent for the drug trial and pharmacokinetic study was obtained from all participants.

Study design

Metoprine was supplied as 2.5, 10, and 50 mg tablets by Wellcome Research Laboratories, Burroughs Wellcome Company (Research Triangle Park, N.C.). Leucovorin was supplied by the Division of Cancer Treatment, National Cancer Institute, as a powder and reconstituted with sterile water to a concentration of 10 mg/ml and given by rapid intravenous injection. Eight pharmacokinetic studies were done in the six patients. Patient 1 received DDMP at 65 mg/m² without citrovorum-factor rescue. Patients 2 to 6 all received citrovorum-factor rescue (leucovorin, folinic acid, 40 mg/m²) 24 h and 96 h after their dose of DDMP. Patients 2 and 3 both received DDMP at 100 mg/m². Patient 4 received 100 mg/m² DDMP followed by a second dose of 175 mg/m² 6 weeks later. Patient 5 received 125 mg/m² DDMP followed by 175 mg/m² DDMP 3 weeks later. Patient 6 received a single dose of DDMP at 175 mg/m². From each patient, 8 ml blood samples were drawn through a heparin lock, centrifuged immediately and the resulting serum refrigerated. Urine collections were also refrigerated as soon as the collection period was ended. Control urine and blood samples were obtained within one hour before the DDMP dose. Each dose of metoprine was given orally with half a glass of water. Blood samples were obtained at 1, 2, 4, 8, 12, 20, 24, 28, 44, 52, 68, 76, 92, 100 and 120 h after the DDMP dose. Urine was collected in 3-h fractions for the first 9 h, and then in 6-h fractions until the end of the fifth day.

Analytical methods

Metoprine for standards was supplied by the Burroughs Wellcome Company, Triangle Park, North Carolina. NADPH was obtained from Boehringer Mannheim, Indianapolis, Indiana, and 2-mercapto-

ethanol from Eastern Organic Chemicals, Rochester, New York. Folic acid used in the preparation of dihydrofolic acid was purchased from K&K Laboratories, Plainview, New York. Dihydrofolate reductase was prepared from frozen rabbit liver acetone powder obtained from Pel-Freez Biological, Inc., Rogers, Arkansas. Metoprine levels were determined by the enzyme inhibition assay of Calvert, Cridland & Harrap (1977), with the following modifications:

(a) Dihydrofolic acid was prepared by the reduction of folic acid with ascorbic acid and sodium hydrosulphite, according to the method of Futterman & Silverman, 1957.

(b) Dihydrofolate reductase was prepared by the following method: 200 mg of rabbit liver acetone powder was weighed and suspended in 10 ml of 0.1 M sodium potassium phosphate buffer (pH 7.0). This was placed on ice for 1 h with intermittent shaking. After centrifugation, the supernatant was frozen; this preparation was stable for 1 month at -5°C .

(c) The components in the microcuvette were as follows:

Sodium potassium phosphate buffer (0.1 M, pH 7.0)	800 μl
β -mercaptoethanol (0.10 M) in 0.01 N HCl	100 μl
Dihydrofolate reductase preparation	100 μl
DDMP stock solution (10 mg/100 ml in 0.01 N HCl; dilutions of stock made up in H ₂ O)	60 μl
NADPH (2.4 mM)	100 μl

All of the samples for a given patient's serum or urine collections were assayed simultaneously. The reproducibility of replicate samples upon reassay was $\pm 10\%$.

This method will detect DDMP and its active metabolites, of which the *N*-oxide has already been identified (Hubbell, Grace & Henning, 1978). The serum and urine activities are therefore expressed as 'metoprine equivalents'. Area under the curve from 0 to 120 h (AUC) was obtained by trapezoidal area analysis performed on a Tektronix 31 terminal.

Only patients with measurable lesions were included in this study, and the following standard definitions of response were used in an attempt to measure changes in tumor volume. A partial remission was defined as a 50% decrease in the sum of the products of the greatest perpendicular diameters of all measurable lesions without evidence of increase in the size of any lesion, or appearance of new lesions. Minor response is defined as an objective ($\geq 25\%$) tumour decrease, but less than that required for a partial remission; no change is a less than 25% change in tumour size and progression is an objective increase greater than 25% in measurable disease or the appearance of new tumour lesions.

Results

The observations of the eight kinetic studies are shown in Figure 2. The serum level of metoprine equivalents ν time curves were irregular and no terminal exponential elimination rate could clearly be observed during this study. For this reason, a model independent analysis of the data was performed.

Peak serum levels of metoprine equivalents and AUC each correlated modestly with the administered dose (Figures 3 and 4). The correlation coefficient for AUC ν dose was 0.68, and for peak serum levels ν dose was 0.64. The AUC and peak serum levels were found to be linearly related to each other with $r = 0.99$, ($P < 0.001$) over the range of doses studied.

Total urinary excretion of dihydrofolate reductase inhibitors (as metoprine equivalents) in the first 120 h ranged from 5 to 17% of the administered dose (Figure 5).

One partial remission and one minor response were seen in the six patients studied (Table 1). The highest peak levels and AUCs were observed in these two patients. The only patient with an AUC $> 200 \mu\text{g ml}^{-1} \text{ h}$ and peak level $> 2.5 \mu\text{g/ml}$ experienced marked thrombocytopenia to $23,000/\text{mm}^3$ in spite of leucovorin rescue, as well as a minor therapeutic response to the drug. The other three patients receiving leucovorin rescue had lower peak levels and AUCs and had neither therapeutic response nor haematologic toxicity.

Discussion

Serum levels of metoprine equivalents ν time were observed to be irregular over a 120 h time period in our studies; AUC showed a better correlation with peak serum levels than either parameter showed separately with dose.

Since the enzyme inhibition assay used to quantitate metoprine can measure metabolites which might have some activity as dihydrofolate reductase inhibitors, the results are expressed as metoprine equivalents. Metoprine is metabolized to at least ten biotransformation products, among which metoprine-1-oxide and its glucuronide conjugate have been identified (Sirotinak *et al.*, 1976). Therefore, the serum level ν time profile may be a composite of more than one molecular species and the curve should not be over-interpreted.

Early clinical trials of metoprine in acute leukaemia were abandoned because of severe and prolonged drug toxicity (Murphy *et al.*, 1954). Since then, Cavallito, Brenckman & Nichol (1978) have quoted a half-life of metoprine in man of 216 h (mean; range 165 to 268 h): this is even greater than pyrimethamine (92 h) and Baker's Antifol (5.3 h), other antifolates not requiring active enzymatic transport for cell entry

Figure 2 Serum concentrations of metoprine equivalents as determined over 120 h following a single oral dose of metoprine. 1 = patient 1; 2 = patient 2; 3 = patient 3; 4,5 = consecutive studies in patient 4; 6,7 = consecutive studies in patient 5; 8 = patient 6.

Figure 3 Area under the curve (AUC) plotted against administered dose; the number correlates with the patient studies in Figure 2.

(Stickney *et al.*, 1973; Hall, Benjamin & Tenczynski, 1978).

We have confirmed the persistence of considerable dihydrofolate reductase inhibitory activity following a single dose of metoprine. Indeed, in our studies of 5 days duration, we failed to observe an exponential terminal elimination phase of metoprine-equivalents in serum, and a final plateau of urinary excretion was only observed in 1 patient (Figure 5, Patient 3).

Administered oral dose is not an adequate predictor of metoprine toxicity nor therapeutic response (Miller *et al.*, 1976; Newman *et al.*, 1979); the variability observed in response to this drug may be explained in part by interindividual differences in metoprine disposition (Newman *et al.*, 1979; Hall *et al.*, 1978). We have used a model-independent analysis, AUC, as an index of total body exposure to the drug and compared this to peak serum level and dose respectively.

Our studies show that the peak level of dihydrofolate reductase inhibitors correlates well with AUC over the first 5 days. In those patients receiving leucovorin rescue, no response nor haematologic toxicity was observed when the peak level was $< 1.6 \mu\text{g/ml}$ and the AUC $< 130 \mu\text{g ml}^{-1} \text{ h}$ ($P < 0.05$).

For drugs with more than one active metabolite, a rigorous correlation of drug level with pharmacological

Figure 4 Peak serum level plotted against administered dose; the numbers correlate with the patient studies in Figure 2.

response is impossible without a detailed knowledge of both the relative potencies and pharmacokinetic characteristics of the individual active metabolites. While the identification and characterization of these metabolites of metoprine may extend over many years, an assay of total pharmacologic activity is available now. We have demonstrated how using this assay and a model-independent analysis of pharmacokinetic data, useful information for understanding interindividual variation in drug response can be obtained.

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Figure 5 Cumulative urinary excretions of metoprine equivalents following a single dose of metoprine; the numbers correlate with the patient studies in Figure 2.

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Table 1 Dosage, clinical response, haematopoietic toxicity, peak serum level of metoprine equivalents and area under the curve (0–120 h) in the eight patient studies

Study*	Response	Haematopoietic toxicity (N = Nadir)	Peak level ($\mu\text{g/ml}$)	AUC _{0–120 h} ($\mu\text{g ml}^{-1} \text{h}$)
Patient 1: 65 mg/m ² (no leucovorin)	Progression	Thrombocytopenia (N = 14,000) Leukopenia (N = 2,100)	0.6	52
Patient 2: 100 mg/m ² (leucovorin)	Progression	No change	1.5	122
Patient 3: 100 mg/m ² (leucovorin)	No change	No change	1.4	108
Patient 4: 100 mg/m ² (leucovorin)	Partial remission	No change	2.1	145
Patient 4: 175 mg/m ² (leucovorin)		No change	2.4	197
Patient 5: 125 mg/m ² (leucovorin)	Minor response	No change	1.8	140
Patient 5: 175 mg/m ² (leucovorin)		WBC: no change Thrombocytopenia (N = 23,000)	2.8	228
Patient 6: 175 mg/m ² (leucovorin)	Progression	No change	1.2	92

*The study column includes the patient number, oral dosage of metoprine and whether or not leucovorin was administered.

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