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Clinical and pharmacokinetic studies of high-dose levamisole in combination with 5-fluorouracil in patients with advanced cancer

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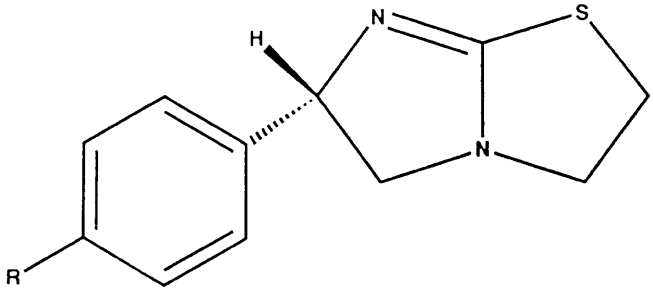
Abstract *Purpose*: To determine the maximum tolerable dose (MTD) and activity of levamisole administered concurrently with 5-fluorouracil (5-FU) in a standard 5-day course. To determine the pharmacokinetics of levamisole during the course of treatment. *Patients and methods*: Levamisole was administered to 38 patients orally three times a day for 5 days concurrently with a course of 5-FU administered daily by rapid intravenous injection for 5 days. Toxicity was evaluated in 20 patients who received escalating doses of levamisole. The activity of the combination was evaluated in 18 patients who received levamisole at the MTD with 5-FU. The pharmacokinetics of levamisole were characterized in ten patients at the MTD level. *Results*: Intractable vomiting, confusion and vertigo were the major dose-limiting toxicities. The MTD of oral levamisole was 100 mg/m^2 administered three times a day concurrently with 450 mg/m^2 per day intravenous 5-FU for 5 consecutive days. Partial responses lasting 5 and 11 months were observed in 2/18 patients with measurable disease at the MTD. Peak plasma concentrations of 1 μg/ml (range 0.6–1.3 μg/ml) were achieved 90 min (range 60–360 min) after an oral dose of 100 mg/m^2 levamisole with a 3.5-fold accumulation noted following 4 days of administration. Peak plasma concentrations of *p*-hydroxylevamisole were about 5% of parent drug. Little parent drug (2–5%) was detected in urine. *Conclusions*: Levamisole may be administered safely with 5-FU at doses which are up to four to five times greater than those presently given in conventional regimens. The recommended dose of levamisole combined with 5-FU for future research protocols is 75 mg/m^2 t.i.d for 5 days.

Key words Levamisole · Toxicity · Pharmacokinetics

Introduction

The combination of 5-fluorouracil (5-FU) and levamisole (Fig. 1) administered following resection of Dukes' C colon carcinoma reduces disease recurrence and enhances survival compared with surgery alone [1, 2]. Although the combination of 5-FU and levamisole has not been compared directly with 5-FU alone, multiple studies over many years have failed to show increased survival with single-agent 5-FU as adjuvant treatment of colon carcinoma [3]. The levamisole dosage regimen adopted for combination with 5-FU was chosen empirically based on the standard regimen used for treatment of human parasite infections, e.g. 50 mg given orally three times daily



R = H: Levamisole

R = OH: *p*-Hydroxylevamisole

R = CH_3: *p*-Methyllevamisole

Fig. 1 Structures of levamisole, *p*-hydroxylevamisole and *p*-methyllevamisole

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for 3 consecutive days and repeated every 2 weeks. The 5-FU regimen consists of five consecutive daily intravenous doses of 450 mg/m^2 followed 4 weeks later by weekly injections of 450 mg/m^2 which are continued for a period of 1 year. A number of unusual toxicities have been seen with this regimen, primarily involving the central nervous system, skin, and joints [1, 4].

Levamisole preferentially potentiates the in vitro antiproliferative activity of 5-FU compared to 5-fluoro-2′-deoxyuridine in a dose-dependent manner [5]. The potentiation of 5-FU antiproliferative activity by levamisole occurs if 5-FU is administered concomitantly and after exposure of cells to levamisole, raising the possibility that levamisole modifies cellular processes in a way that cells become more vulnerable to one or more of the various biological activities of 5-FU. *p*-Hydroxylevamisole, a levamisole metabolite, has approximately the same potency of potentiation of 5-FU antiproliferative activity as the parent drug [6]. Because levamisole is known to inhibit a variety of alkaline phosphatases, the ability of phosphatase inhibitors chemically unrelated to levamisole to potentiate the inhibition of in vitro human tumor cell growth inhibition by 5-FU was investigated. Orthovanadate, an inhibitor of tyrosine phophatases, potentiates the inhibitory activity of 5-FU against the same tumor cell lines [7].

There are few published reports on the clinical pharmacology of levamisole and its metabolites in human subjects. The distribution and elimination of levamisole are similar in healthy subjects and patients [7]. Following a single 2–5 mg/kg oral dose, peak plasma concentrations of 0.7–1.7 μg/ml are observed within 1.5 h of drug administration and the elimination half-life of levamisole is 4–5.5 h. There are no data available on the plasma disposition of levamisole during a multiple dose regimen. Although levamisole is extensively metabolized in vivo [8] the only documented metabolites in humans are *p*-hydroxylevamisole and its glucuronide metabolite which have been detected in urine.

Since levamisole potentiates 5-FU in vitro antiproliferative activity in a concentration-dependent fashion, we initiated a phase I trial of escalating levamisole doses administered with daily doses of 5-FU over the full 5-day chemotherapy course. The objectives were to determine the maximum tolerable dose (MTD) of levamisole that could be administered with 5-FU without encountering added toxicity to the degree that it would be prohibitive as surgical adjuvant treatment in colon cancer, and to characterize the pharmacokinetics of levamisole and *p*-hydroxylevamisole during repeated dosing of levamisole.

Materials and methods

Patient eligibility

Patients selected for this study had histologically confirmed, unresectable, advanced cancer, ECOG performance status ≤ 1, a white blood cell (WBC) count ≥ 4000/mm^3 and a platelet count ≥ 100 000/mm^3. Patients were excluded if they had received prior chemotherapy for unresectable disease or radiation therapy or had experienced recurrence while on 5-FU and levamisole therapy or within the 6 months of completion of the treatment. Patients were also excluded if they had an active infection, frequent vomiting, severe anorexia or recent major surgery. Written informed consent was required.

Patient follow-up

Baseline evaluation of patients enrolled in the study included a complete history and physical examination, complete blood count, chemistry group, chest radiograph and a serum pregnancy test in women of child-bearing potential. WBC and platelet counts were repeated weekly during the interval between treatment cycles. Physical examination, complete blood count, platelet count, hemoglobin determination and chemistry group were obtained on day 6 of the first course and prior to subsequent treatment cycles.

Treatment plan

5-FU was given by rapid intravenous (i.v.) injection daily for 5 days at a fixed dose of 450 mg/m^2. Levamisole was given orally three times a day for 5 days, with the morning dose preceding the 5-FU injection. Treatment was repeated every 5 weeks until disease progression. The starting dose of levamisole was 25 mg/m^2 t.i.d. (level 1). Patients were entered in groups of four at each levamisole dose level. New patients were entered at the next higher dose level only after at least three of four patients at the previous level had been observed for a minimum of 5 weeks. The levamisole dose was escalated as follows: level 2, 50 mg/m^2 t.i.d.; level 3, 75 mg/m^2 t.i.d.; level 4, 100 mg/m^2 t.i.d.; level 5, 150 mg/m^2 t.i.d.; level 6, 225 mg/m^2 t.i.d.; level 7, 300 mg/m^2 t.i.d.

The MTD was defined as the highest dose of levamisole which would result in severe leukopenia (WBC < 2000/mm^3) in ≤ 30% of patients when given with 5-FU 450 mg/m^2 i.v. for 5 days. Dose-limiting toxicity (DLT) was leukopenia (WBC < 2000/mm^3) in > 30% of patients, or the presence of other intolerable toxicity. If two or more patients on any level experienced a WBC nadir of < 2000/mm^2, then three additional patients were added (depending on global evaluation of toxicity at that level). Dose escalation was discontinued if three or more of seven patients experienced WBC < 2000/mm^3, and additional patients were entered at the next lower dose level. A final expansion of patient entries to 20 at a given levamisole dose level was carried out to more adequately define a dose that produced leukopenia (WBC < 2000/mm^3) in ≤ 30% of patients and with no substantive incidence of any other major toxic reaction.

Response evaluation (for patients with measurable indicator lesions)

Measurable disease was defined as a lesion that could be clearly measured in two dimensions on chest radiograph, CT scan, or physical examination. Hepatomegaly was allowed as measurable disease only if the liver was proved to contain metastasis and if a clearly palpable liver edge was felt at least 5 cm below the xyphoid process or costal margins on quiet respiration. A positive CT or isotope liver scan could be used if there was a clearly defined perfusion defect measuring at least 5 cm in greatest diameter.

A complete response was defined as clearing of all detectable malignant disease. A partial response was defined as a > 50% reduction in the product of the longest perpendicular diameters of the primary indicator lesion. If malignant hepatomegaly was the indicator lesion, a > 30% decrease in the sum of liver measurements below the xyphoid process and below each costal margin at the midclavicular lines was required. An increase in the size of any other area of malignant disease, the development of new lesions,

worsening of performance status and weight loss exceeding 10% were criteria for progression.

Pharmacologic studies

Chemicals

Levamisole and 5-FU were obtained from Sigma Chemical Co. (St. Louis, Mo.). *p*-Hydroxylevamisole and *p*-methyllevamisole were generous gifts from Janssen Research Foundation (Spring House, Pa). HPLC grade solvents were obtained from Fisher Scientific (St. Louis, Mo.).

Specimen collection

On day 1 and day 4 or 5, blood specimens were obtained prior to the first oral dose of levamisole on that day and at the following times after administration: 20, 40, 60, 90, 120, 240 and 360 min. In addition, a single sample was obtained prior to the first oral levamisole dose on days 2, 3 and 4 or 5 (days during which full plasma sampling was not performed). Blood samples (7 ml) were collected in heparinized tubes and immediately chilled, and the plasma was separated by low-speed centrifugation. Plasma samples were transferred to plastic tubes and stored at −20 °C until analysis.

Urine was collected in separate plastic containers during the 8-h period following each levamisole dose on day 1 and on day 4 or 5 (the day of the second complete set of blood samples).

Levamisole analysis

Following the addition of *p*-methyllevamisole (100 ng), samples were extracted using the method of Kouassi et al. [9]. The residue obtained by evaporation of the organic phase was reconstituted in methanol (50 µl) and 2 µl was injected into a gas chromatograph. Gas chromatography was performed on a Hewlett-Packard Model 5840A chromatograph equipped with a nitrogen-phosphorus detector and a glass column (120 cm × 2 mm i.d.) containing 3% SP-2250 DB on 100/120 mesh Supelcoport. The oven temperature was 220 °C and the injector and detector temperatures were 290 °C and 340 °C, respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min.

p-Hydroxylevamisole analysis

Patient or control plasma samples (1 ml) were extracted by solid-phase extraction with 1-ml Baker C_{18} SPE columns (Phillipsburg, N.J). Prior to sample addition, columns were each rinsed with 2 ml methanol and distilled water. Samples were next eluted through the columns, the adsorbed materials were washed with 2 ml distilled water and *p*-hydroxylevamisole was eluted with 1 ml methanol. Solvent was evaporated under a gentle stream of nitrogen and the residue reconstituted in 100 µl mobile phase for HPLC analysis.

HPLC separation was achieved on an IBM C_8 reverse-phase column (250 mm × 4.6 mm i.d.) with a mobile phase consisting of acetonitrile/50 m*M* potassium phosphate, pH 4.5 (50:50) at a flow rate of 1 ml/min. The HPLC system consisted of Shimadzu components including a model LC6A pump, a model SCL6B autosampler, a model SPD6AV UV absorbance detector and a model CR4A computer to acquire and integrate data. Reconstituted extracts (20 µl) were injected and the column effluent was monitored at 230 nm.

Data analysis

The elimination rate constant (k_{el}), AUC and plasma clearance were determined by noncompartmental methods as described by Gibaldi and Perrier [10]. The value of k_{el} was calculated by linear least squares regression from the slope of the terminal exponential phase of the graph of ln(plasma concentration) versus time. The AUC was determined by a trapezoidal approximation from zero time to the time of the last detectable plasma concentration (C_{last}) with residual area after C_{last} calculated as $AUC_r = C_{last}/K_{el}$. Oral plasma clearance was calculated as Cl_p/F = dose/AUC, where F is the oral bioavailability. The elimination half-life was calculated as $t_{1/2} = 0.693/k_{el}$.

Results

A total of 38 patients were entered into the study, of whom 20 were enrolled during the dose escalation phase of the study and an additional 18 were enrolled at the MTD. The characteristics of the patients are shown in Table 1. The patients had an ECOG performance status of 0 or 1. The majority of the patients had not received prior treatment.

Toxicity

During the dose escalation, the MTD of oral levamisole was 100 mg/m^2 t.i.d. for 5 days when given with 5-FU (450 mg/m^2 per day for 5 days). Four of five patients treated at this level completed their first course of treatment with levamisole and 5-FU. In one patient, the levamisole dose on the 5th day of the fourth course of treatment was reduced 50% because of nausea and vomiting. This patient also required fluids periodically during the four treatment courses. Three patients who received two or more courses of treatment required a reduction of their 5-FU dose because of myelosuppression.

Table 1 Summary of patient characteristics

	Phase I	Phase II
No. of patients:	20	18
Sex (male:female):	13:7	8:10
Age (years)		
Median	62	60
Range	43–82	49–76
Previous treatment	Chemotherapy (5), radiation therapy (3), none (12)	Chemotherapy (3), radiation therapy (1), none (15)
ECOG performance status	0 (6), 1 (14)	0 (10), 1 (8)
Diagnosis	Colon (4), pancreas (4), unknown (3), gastric (3), bile duct (3), miscellaneous (3)	Colon (13), gastric (2), rectum (1), pancreas (1), gastroesophageal junction (1)

Table 2 Number of treatment cycles associated with moderate to severe (grade 2–3) toxicity (other toxicities: 50 mg/m^2 – malaise, 75 mg/m^2 – rash, 100 mg/m^2 – alopecia, 150 mg/m^2 – confusion)

Dose (mg/m^2 t.i.d. ×5)	Patients/ Cycles (no.)	Stomatitis	Diarrhea	Nausea	Vomiting	Anorexia	Mood change	Metallic taste	WBC (grade 3–4)	PLT
25	4/23	–	–	–	–	–	–	–	1	1
50	4/21	3	4	6	2	3	–	–	3	–
75	4/36	–	2	–	–	–	–	–	2	–
100	5/11	–	–	1	–	3	1	1	4	–
150	3/3	1	1	3	3	1	1	1	1	–
(150 → 100	2/13	–	–	4	1	1	–	–	1	–)
Total	20/94	4	7	10	5	7	2	2	11	1

At doses of 100 and 150 mg/m^2 levamisole, patients complained of a metallic taste in the mouth which was unpleasant but not intolerable. There was no clear relationship between dose of levamisole and grade 3 or 4 leukopenia over the range of 25–150 mg/m^2 t.i.d. for 5 days (Table 2).

Clinically intolerable toxicity occurred when levamisole 150 mg/m^2 t.i.d. for 5 days was given with 5-FU at 450 mg/m^2 per day for 5 days. The major toxicities seen at this dose were intractable vomiting, confusion and vertigo (Table 2). None of the three patients treated at this dose level was able to complete the 5-day course of therapy. Two of the patients treated with an initial course of levamisole 150 mg/m^2 t.i.d. for 5 days were treated with a lower dose of levamisole (100 mg/m^2 t.i.d. for 5 days) in subsequent courses. One of those patients completed nine courses of levamisole 100 mg/m^2 t.i.d. for 5 days without intolerable toxicity. The other patient completed two of four courses of levamisole 100 mg/m^2 t.i.d for 5 days. This patient did not receive treatment on day 5 of the second course and refused further treatment after day 4 of the fifth course. This patient also received additional fluids on day 4 of course 3.

When patient entries were expanded to further evaluate toxicity, 18 patients received a total of 64 cycles of treatment with levamisole 100 mg/m^2 t.i.d. for 5 days in combination with 5-FU 450 mg/m^2 per day for 5 days. The median number of cycles was 2 with a range of 1–12. Drug toxicity was moderate and most commonly consisted of leukopenia or gastrointestinal symptoms (Table 3). Hematologic toxicity was limited to leukopenia. This occurred in 53% of treatment courses and was severe (grade III or more) in 11%. Thrombocytopenia was mild. Nausea occurred often (72% of the administered courses) and was severe in 8%. Vomiting occurred in 41% of treatment courses, but was severe in only 3%. Other toxicities with an incidence greater than 25% were stomatitis, diarrhea, alopecia and anorexia. However, their manifestation as severe toxicities was low (≤ 3%) with the exception of stomatitis which was severe in approximately 6% of the treatment cycles. The incidence of mood change, smell and taste change and rash was low (< 20%) and these symptoms were not severe. Other toxicities (Table 3) were rare with incidences < 5%.

Response

Only one patient treated during the dose escalation phase of the study had measurable disease. This patient with metastatic colon carcinoma had a partial response following the first cycle of treatment lasting for approximately 7 months. A second patient with metastatic gastric cancer had regression of non-measurable (abdominal) disease noted 5 weeks following the second course of therapy lasting for approximately 17 months.

Table 3 Percentage of patients with toxic reactions in the expanded cohort (n = 18). Treated with combined levamisole (100 mg/m^2 t.i.d. × 5 days) and 5-FU (450 mg/m^2/d × 5 days)

Hematologic toxicity		
Leukopenia		
< 4000/μl	53	
< 2000/μl	11	
Thrombocytopenia		
< 130000/μl	12	
< 50000/μl	0	
Nonhematologic toxicity	**Any**	**Grades 3 or 4**
Stomatitis	26	6
Diarrhea	30	3
Nausea	72	8
Vomiting	41	3
Anorrexia	27	3
Alopecia	28	2
Mood change	11	0
Smell and taste change	19	0
Rash	9	0

Of 18 patients in the expanded cohort, 15 were eligible for evaluation of objective tumor response. Three patients were unevaluable owing to the absence of a measurable indicator lesion. Two patients had partial responses lasting 5 and 11 months (one with colon cancer, and one with stomach cancer), 11 had stable disease with a median duration of 4 months (range 2–15 months), and 2 had progressive disease after their initial course of 5-FU/levamisole.

Drug analysis

Efficient separation of levamisole and *p*-methyllevamisole was achieved by gas chromatography through a glass column packed with 3% SP-2250 DB on 100/120 mesh Supelcoport. Levamisole and *p*-methyllevamisole were isolated from plasma by the back extraction technique developed by Kouassi et al. [9]. The lower limit of quantitation and linear range of the levamisole standard curves were 0.05 μg/ml and 0.05–2.0 μg/ml, respectively. Day-to-day assay reproducibility, based on the coefficient of variation of the slope, was 8.5% ($n = 10$).

Kouassi et al. also described an assay for the determination of *p*-hydroxylevamisole in urine, but the sensitivity of this assay is insufficient to detect this levamisole metabolite in plasma. Solid phase extraction through a C_{18} column provided a rapid and efficient method to isolate *p*-hydroxylevamisole from plasma. Interference-free chromatograms of *p*-hydroxylevamisole were achieved following reverse-phase chromatography of the reconstituted extracts on an octyl column and UV detection at 230 nm. The lower limit of quantitation and linear range ($r^2 > 0.99$) of *p*-hydroxylevamisole standard curves were 10 ng/ml and 10–200 ng/ml. Day-to-day assay reproducibility, based on the coefficient of variation of the slope, was 4.6% ($n = 9$).

Pharmacologic investigations

The pharmacokinetics of levamisole and *p*-hydroxylevamisole were investigated in ten patients who received oral levamisole 100 mg/m^2 t.i.d. for 5 days in combination with 5-FU 450 mg/m^2 per day for 5 days. Full pharmacokinetics were determined on day 1 of levamisole administration in all ten patients and were repeated on day 4 in one patient and on day 5 in four patients. In addition, trough plasma samples were obtained from each patient immediately before each daily dose of 5-FU. Representative plasma profiles of levamisole and *p*-hydroxylevamisole during the 5-day regimen are illustrated in Fig. 2. Following the first oral dose, the peak plasma concentration of 1 μg/ml levamisole was achieved 120 min after drug administration and the mean elimination half-life was 241 min (Table 4). The mean (±SD) AUC and apparent oral clearance values were 439 (±395) μg/ml min and 351 (±129) ml/min per m^2, respectively.

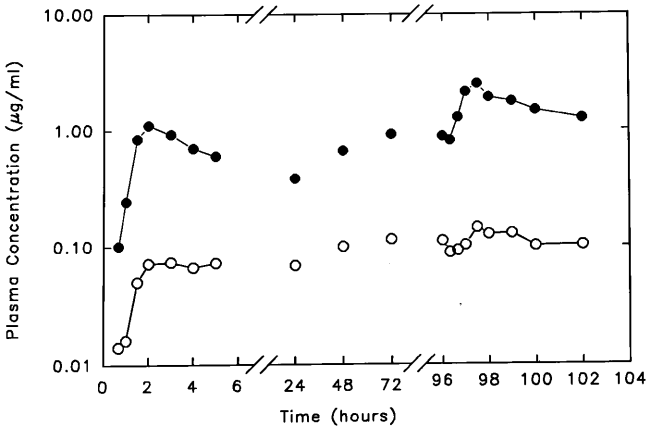

Fig. 2 Representative plasma profile of levamisole (*closed circles*) and *p*-hydroxylevamisole (*open circles*) for a patient (patient 1) treated with levamisole, 100 mg/m^2 t.i.d. for 5 days

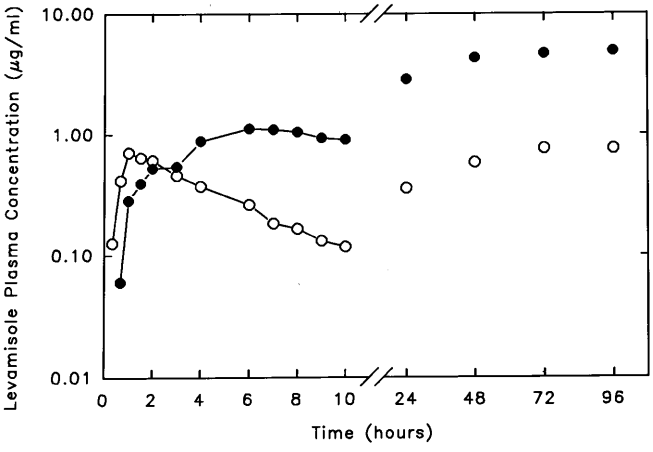

Fig. 3 Plasma profile of levamisole in the patient (patient 8) with severe liver disease (*closed circles*) compared with a patient (patient 7) with normal liver function (*open circles*). Both patients were treated with levamisole 100 mg/m^2 t.i.d. for 5 days

The peak plasma concentration of levamisole increased to 2.45 μg/ml and the mean elimination half-life increased to 477 min when pharmacokinetics were examined on day 4 (one patient) and day 5 (four patients) (Table 5). The levamisole accumulation factor (R) of 3.45 obtained by comparison of the day 1 and day 5 peak plasma concentration data was greater than the value expected based on the day-1 elimination half-life (theoretical R value, 1.22). Therefore, the second oral dose of levamisole on day 1 was delayed and blood sampling was prolonged to 10 h in four patients to better characterize levamisole elimination. In three of the four additional patients studied, the levamisole peak plasma concentrations and elimination half-lives were similar to those observed for the first six patients (Table 4). The levamisole elimination half-life for one patient was substantially delayed as described in detail below.

The peak plasma concentration of *p*-hydroxylevamisole (0.05 μg/ml) was approximately 5% of the value

Table 4 Individual pharmacokinetic parameters for patients on day 1 of treatment with 100 mg/m^2 levamisole (t.i.d. × 5 days) (ND not detected)

Patient	Levamisole			*p*-Hydroxylevamisole		
	t_{max} (min)	C_{max} (μg/ml)	$t_{1/2}$, el (min)	t_{max} (min)	C_{max} (μg/ml)	$T_{1/2}$, el (min)
1	120	1.11	194	180	0.074	> 1000
2	90	0.76	207	90	0.027	317
3	90	1.09	217			
4	90	1.27	508	90	0.048	411
5	180	1.08	184	360	0.052	560
6	60	0.72	135	180	0.065	268
7[a]	60	0.71	206	90	0.067	502
8[a]	360	1.10	672	ND		
9[a]	90	0.80	183	120	0.085	428
10[a]	120	0.62	174	120	0.032	141
Mean (SD)	126 (89)	1.03 (0.43)	268 (176)	154 (91)	0.056 (0.020)	375 (144)

[a] The second day 1 levamisole dose was delayed to obtain blood samples for 10 h. Pharmacokinetics were not repeated on day 4 or day 5

Table 5 Individual pharmacokinetic parameters for patients on day 4 or day 5 of treatment with 100 mg/m^2 levamisole (t.i.d. × 5 days)

Patient	Levamisole			*p*-Hydroxylevamisole		
	t_{max} (min)	C_{max} (μg/ml)	$t_{1/2}$, el (min)	t_{max} (min)	C_{max} (μg/ml)	$T_{1/2}$, el (min)
1	90	2.50	383	90	0.146	535
2[a]	120	1.16	288	90	0.058	347
3	90	2.94	518			
4	120	2.45	400	120	0.079	450
6	120	1.92	607	240	0.077	564
Mean (SD)	105 (17)	2.45 (0.42)	477 (105)	150 (79)	0.101 (0.039)	516 (59)

[a] Complete set of plasma samples day 4 instead of day 5

ascribed to the parent drug. The time to achieve peak plasma concentration (180 min) and the apparent elimination half-life (375 min) were delayed compared with parent drug (Table 4). The mean peak plasma concentration (0.10 μg/ml) and elimination half-life (516 min) were greater compared with day 1 when pharmacokinetic studies were repeated on day 4 or day 5 (Table 5).

Interestingly, levamisole plasma distribution and elimination was greatly delayed in one patient (patient 8) with severe liver abnormalities consistent with biliary obstruction (direct bilirubin 12.9 mg/dl, total bilirubin 17.4 mg/dl, alkaline phosphatase 1248 U/l, SGOT 172 U/l) who deteriorated rapidly during the course of therapy. Physical examination showed significant hepatomegaly suggesting extensive intrahepatic metastases. The patient had progressive liver failure and developed hepatic encephalopathy. The peak plasma concentration of levamisole (1.1 μg/ml) was observed 6 h after oral administration and the apparent elimination half-life was greater than 11 h. The trough value of levamisole on day 5 was 5 μg/ml compared with 0.4–2.3 μg/ml in patients with normal liver function. The plasma levamisole concentration remained elevated (3.8 μg/ml) 2 days after the end of treatment. Furthermore, *p*-hydroxylevamisole was not detected in plasma samples from this patient.

Approximately 2–5% of the levamisole dose was recovered in the urine during the first day of drug administration. Urinary recovery values were similar when collection was repeated on day 4 or 5.

Discussion

The MTD of oral levamisole was 100 mg/m^2 t.i.d. for 5 days with 5-FU (450 mg/m^2 per day for 5 days). The DLT was nausea and vomiting. This dose of levamisole was approximately *five times* the total amount of levamisole given in the standard 5-FU/levamisole regimen as "adjuvant" therapy following resection of Dukes' C colon carcinomas.

The addition of levamisole to 5-FU did not enhance significantly the myelosuppression associated with 5-FU. Levamisole seemed to increase the GI toxicity, anorexia, nausea and vomiting and occasional diarrhea that occurred with the same regimen of 5-FU alone. Escalation of levamisole to 150 mg/m^2 t.i.d. for 5 days resulted in significant central nervous system toxicity and severe vomiting.

Despite the striking beneficial effect of levamisole combined with 5-FU in patients with Duke's C carcinoma, the pharmacology of this combination has been

poorly described. There are few published reports on the clinical pharmacology of levamisole [7, 9, 11] and only one report on levamisole pharmacokinetics in cancer patients [7]. We examined the plasma distribution and elimination of levamisole and *p*-hydroxylevamisole in patients who received combined levamisole/5-FU as adjuvant treatment for resectable colorectal cancer. Pharmacokinetic parameters of levamisole determined for this small group of patients were similar to those observed for healthy individuals and cancer patients who received a single 2.5 mg/kg ($\sim$100 mg/m^2) oral dose of levamisole. The peak plasma concentration of 1.0 μg/ml was observed approximately 1.5 h after drug administration and the elimination half-life of levamisole was 4–5.5 h. The peak plasma concentrations of levamisole in patients were far below the in vitro concentration (90 μg/ml) that potentiates the antiproliferative effect of 5-FU. This observation suggests that tumors accumulate levamisole or that levamisole metabolites may also potentiate the antiproliferative effect of 5-FU. Equipotence of *p*-hydroxylevamisole with the parent drug [6] is consistent with the latter proposition. Although *p*-hydroxylevamisole plasma concentrations were lower than those of parent drug, other levamisole metabolities may have activity.

Several observations from our study suggest that the liver plays an important role in the plasma clearance of levamisole. Little parent drug was excreted in the 24-h urine, levamisole plasma accumulation was greater than expected given the 4-h elimination half-life, and a patient with severe impairment of liver function had high (5 μg/ml vs 1 μg/ml) plasma concentrations of levamisole. The low urinary excretion of parent drug observed in our study was similar to the low urinary excretion previously reported for healthy subjects [8, 9, 12]. Most of the administered dose is excreted as metabolites, since approximately 70% of the radioactivity administered in an oral dose of radiolabelled levamisole is excreted in the urine [12], although few levamisole metabolites have been identified in humans. Kouassi et al. [9] found *p*-hydroxylevamisole and its glucuronide conjugate, which accounted for 2% and 10%, respectively, of the total dose of levamisole, excreted in the 24-h urine. We were unable to detect either metabolite in urine, but found low concentrations in the plasma of nine of ten subjects. In contrast, animal studies have shown as many as 15 levamisole metabolites in urine. Clearly, several pathways of metabolism contribute to levamisole clearance.

The 2.5-fold plasma accumulation of levamisole during the 5-day administration schedule was associated with a modest decrease in levamisole elimination on the 5th day of drug administration. We explored the possibility that accumulation was caused by a second, prolonged elimination phase by monitoring levamisole plasma concentrations for a 10-h period following the first dose. However, the elimination half-life values for the two groups of patients were similar. The terminal elimination half-life value of approximately 5 h observed in other pharmacokinetic studies of levamisole also would not predict the extent of accumulation we observed in our study [8, 9, 12]. The reason for levamisole accumulation observed in our study is presently unknown, but it may be associated with alterations in hepatic function that occur during treatment with levamisole and 5-FU. Moertel et al. observed hepatotoxicity in 40% of patients who received levamisole with 5-FU [13]. Toxicity occurred 1–12 months (median 2.5 months) after the onset of therapy, was characterized most often by mild and reversible elevations in alkaline phosphatase and was frequently associated with mild elevations in transaminase and bilirubin. Similar toxicity is uncommon for patients treated with levamisole or 5-FU alone (reference 13 and references cited therein). Since the total dose of levamisole in our study was five- to sixfold greater than the conventional dose utilized in the study by Moertel et al., accumulation may reflect earlier onset of changes in liver function as a result of treatment with the combination of levamisole and 5-FU.

Severe impairment of hepatic function in one study patient was associated with substantially elevated (5 μg/ml vs 1 μg/ml) plasma concentrations and prolonged elimination of levamisole and no detectable *p*-hydroxylevamisole. Since this patient had no toxicity, we speculate that gastrointestinal and central nervous system toxicities of levamisole regimens are caused by levamisole metabolites other than *p*-hydroxylevamisole and not the parent compound. Alternatively, some levamisole metabolites may contribute to the therapeutic benefit of this regimen. Kovach et al. have demonstrated that the metabolite, *p*-hydroxylevamisole, potentiates the antiproliferative activity of 5-FU in vitro comparable with levamisole [6]. Therefore, the efficacy of levamisole regimens may be due to the relative contributions of different metabolic pathways.

Because up to five or six times as much levamisole can be administered safely as has been used in association with 5-FU in conventional regimens, we are evaluating a "high-dose levamisole" regimen combined with 5-FU and leucovorin as surgical adjuvant therapy for high-risk colon cancer. Preliminary experience with levamisole 100 mg/m^2 t.i.d. for 5 days with 5-FU and leucovorin in the current surgical adjuvant protocol (INT 0135) coordinated by the North Central Cancer Treatment Group, has indicated severe neurocortical toxicity (confusion, somnolence, agitation) in approximately 20% of patients, particularly in patients more than 70 years old. It is possible that the addition of leucovorin potentiates the toxicity of this regimen. A reduction of the levamisole dose to 75 mg/m^2 t.i.d. for 5 days in this regimen has resulted in a lower incidence (4%) of severe neurocortical toxicity.

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