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ORIGINAL ARTICLE

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Pharmacokinetics of levamisole in cancer patients treated with 5-fluorouracil

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Abstract *Purpose*: To investigate the pharmacokinetics of levamisole and a metabolite, *p*-hydroxylevamisole in patients with colorectal cancer treated with 5-fluorouracil (5-FU). *Methods*: Following an intravenous bolus dose of 5-FU, 20 patients with colorectal cancer received oral doses of 50 mg levamisole every 8 h for 3 days. Immediately after the last dose, blood and urine samples were collected over at least an 8-h period. Samples were assayed for levamisole and *p*-hydroxylevamisole by GC/MS. The levamisole plasma and urine data were subjected to pharmacokinetic analysis using NONMEM software. *Results*: Substantial interpatient variability was observed in the levamisole plasma concentration-time curves. Patients with cardiovascular or gastrointestinal complications demonstrated altered absorption of levamisole. Pharmacokinetic parameter values for levamisole were similar to those obtained previously in healthy subjects and other cancer patients. *Conclusions*: There is no evidence that the pharmacokinetics of levamisole are altered by 5-FU administered immediately prior to levamisole administration. The relationship between the substantial intersubject variability in levamisole plasma concentration-time curves and clinical outcome following 5-FU/levamisole adjuvant chemotherapy should be examined.

Key words Levamisole · Pharmacokinetics · 5-Fluorouracil · Colorectal · Cancer

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Introduction

Colorectal cancer is a significant health problem and a major cause of death in North America and Europe [10]. In many patients surgical resection of the tumor is possible. With locally advanced disease, however, i.e. transmural or lymph node involvement, this procedure is associated with a high risk of relapse [10]. Improvements in recurrence-free and overall survival have been reported in patients receiving 5-fluorouracil (5-FU) regimens that include the anthelmintic drug levamisole [8]. Moertel et al. have confirmed that this combination reduces the risk of cancer recurrence in patients with Dukes' C disease by 41% and reduces death rate by 33% [12]. Based on these studies, the NCI has advised that 5-FU and levamisole combined should be considered as a standard therapy option for Dukes' C colon carcinoma [13].

The dose and schedule of levamisole used in these studies appears to have been chosen arbitrarily from experience in using the drug as an anthelmintic [12]. However, levamisole has shown both dose-dependent effects on alkaline phosphatase inhibition and immunomodulatory effects [3]. Recently, when used in combination with 5-FU and leucovorin, trends in toxicity have been observed with increasing doses of levamisole [3]. It is possible that the therapeutic effect of levamisole in combination with 5-FU is likewise dose-dependent.

Dose-dependency of effects suggests that the value of the levamisole concentrations in the blood following oral administration may also influence toxicity and therapeutic outcomes. In this regard, the pharmacokinetics of the drug may be of importance. There are a limited number of reports describing the pharmacokinetics of levamisole in humans. Since the pharmacokinetics have only been investigated following oral administration, the fraction of an oral levamisole dose that is absorbed is unknown. Drug absorption is rapid and distribution to the tissues appears to be extensive.

Only 5% of the drug is recovered unchanged in the urine, the balance presumably eliminated by metabolism consistent with animal experiments [15, 16]. The only metabolites identified in humans to date are *p*-hydroxylevamisole and its glucuronide conjugate. The *p*-hydroxy metabolite has been assessed as equivalent to the parent drug in terms of potency in modulating the antiproliferative activity of 5-FU [7] so that its pharmacokinetics are of potential interest. However, urinary recovery of this metabolite is reported to be only 2–5% of that of the parent drug with an additional 10% appearing as its glucuronide conjugate [1, 5, 6].

A well-recognized problem in relating the dose of drugs used in the treatment of cancer to therapeutic or toxic effects is the marked intersubject variability in the pharmacokinetics of these agents [20]. Thus lack of predictability in response may be a result of widely differing plasma drug concentrations among patients receiving identical doses. If this indeed was true for levamisole then, since the drug is administered on a chronic basis, dose adjustment based on initial blood levels could be used to optimize the regimen. The objectives of this study were (1) to characterize the pharmacokinetics of levamisole and *p*-hydroxylevamisole in patients undergoing adjuvant therapy with 5-FU and levamisole and (2) to assess the extent and source of interpatient variability of the pharmacokinetics of levamisole when given in combination with 5-FU.

Material and methods

The study was approved by the UNMC Institutional Review Board (IRB). All patients signed an informed consent form.

Subjects and study design

The study group comprised 20 patients, (9 males and 11 females, age 58.3 ± 10.0 years, weight 78.5 ± 13.5 kg, means ± standard deviation). Eligible patients were referred to the study by UNMC and regional oncologists. One patient was Hispanic, one a Native American and the remainder Caucasian. One patient was excluded from subsequent analysis due to failure to freeze plasma samples.

Patients had histologically confirmed Dukes' C adenocarcinoma of the colon, had successfully completed the initial phase of adjuvant therapy with levamisole and 5-FU and were in the maintenance phase of adjuvant therapy. Patients with concurrent medical conditions which might have interfered with the study, e.g. hepatic and renal dysfunction (i.e. bilirubin >2.0 mg/l, SGOT more than twice the upper limit of normal, serum creatinine >1.5 mg/l) were excluded. Patients taking medication that could likewise affect levamisole pharmacokinetics, e.g. known drug-metabolizing enzyme inducers and inhibitors, were also excluded.

At 8 a.m. of the first day of the study, patients received 450 mg/m^2 5-FU by intravenous bolus injection. Immediately following 5-FU administration, patients ingested a 50-mg levamisole tablet. Patients were given nine doses over 3 days. At 8 a.m., exactly 48 h after the first levamisole dose, the final dose was ingested on a fasted stomach. Blood samples (10 ml) were then drawn at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, and 8 h. During the same time period urine samples were collected and the volumes recorded from 0–2, 2–4, 4–6, and 6–8 h.

Levamisole plasma and urine assay

Plasma and urine were assayed for levamisole and *p*-hydroxylevamisole by a GC/MS assay based upon a method described by Woestenborghs et al. [19]. Using 1 ml of plasma or urine, levamisole was analyzed by a high-resolution GC/MS system (AutospecUltima, Fisons Instruments) using pentadeuterated levamisole as an internal standard. The mass spectrometer was used in the selected ion recording (SIR) mode to monitor the ion current intensities at m/z 204.0720 (levamisole), 292.1066 (*p*-hydroxylevamisole, TMSi) and 209.1035 (internal standard). Using a 15 m × 0.53 mm I.D. OV-1 capillary column (film thickness, 0.88 μm), a column temperature programmed (20 °C/min) from 240 °C to 265 °C and helium as the carrier gas, the retention time was approximately 1 min for levamisole and d_5-levamisole and 2 min for *p*-hydroxylevamisole, TMSi. The limit of quantification for levamisole and *p*-hydroxylevamisole in plasma was 1.0 ng/ml for each compound and in urine 50 and 20 ng/ml, respectively. The accuracy for levamisole and *p*-hydroxylevamisole in plasma was between 96.7% and 105.1% and 93.8% and 103.8% over a range of 1–1000 ng/ml. Corresponding values for urine were similar. Between-assay precision for levamisole in plasma (6.27–396 ng/ml) and *p*-hydroxylevamisole (3.18–201 ng/ml) were 3.6–4.4% and 9.2–12.2%, respectively. Corresponding values for urine were similar. Within-assay precision for levamisole and *p*-hydroxylevamisole in plasma were 2.3–2.7% and 6.0–8.2%, respectively. Corresponding values for urine were similar.

Pharmacokinetic analysis

A pharmacokinetic model that included plasma and urine concentrations of *p*-hydroxylevamisole as well as those of levamisole was examined. The model incorporated the following components. Levamisole is absorbed from the gastrointestinal tract by a first-order process (Ka) and exhibits a prominent absorption lag-time (t_0). It is excreted into the urine ($Cl_{L,R}$) and converted to *p*-hydroxylevamisole ($Cl_{L,OH}$). In addition levamisole is transformed to other metabolites ($CL_{L,M}$). Hydroxylevamisole is similarly excreted into the urine ($CL_{OH,R}$) and converted to other metabolites ($CL_{OH,M}$) such as the glucuronide.

A plot of plasma levamisole and *p*-hydroxylevamisole (not shown) demonstrates that at later times the slopes of the log concentration-time plots of drug and metabolite are parallel. This indicates that the elimination of the metabolite is significantly faster than that of the parent drug.

Animal experiments have demonstrated the existence of at least 15 metabolites of levamisole and its *p*-hydroxy product [15, 16]. A detailed pharmacokinetic model accounting for all elimination pathways of the drug and metabolite is not practical and a simpler model must suffice. The parameter values reported in Table 1 were therefore restricted to those pharmacokinetic parameters that could be assessed with confidence. Specifically, if it was assumed, as is general practice, that the absorption rate constant of levamisole was greater than the elimination rate constant. Then Ka could be estimated along with a lag time. The renal clearance of both parent drug and the hydroxy metabolite could be confidently determined since both plasma and urine measures were available for both compounds. Finally, levamisole non-renal clearance relative to the fraction of the oral dose absorbed, F, could also be determined ($[Cl_{L,OH} + CL_{L,M}]/F$).

Plasma levamisole and *p*-hydroxylevamisole concentrations and urine amounts were analyzed by a population pharmacokinetic method using the software package NONMEM, version V [14]. This program estimates population mean pharmacokinetic parameters as well as inter- and intrasubject variability. It is also capable of evaluating quantitative relationships between pharmacokinetic parameters and patient- and study-specific variables. The pharmacokinetic model selected was a one-compartment model for levamisole and *p*-hydroxylevamisole with first-order levamisole absorption, urine output compartments plus non-renal elimination pathways. A two-compartment model did not produce a superior fit to the data.

Table 1 Estimates for population parameters for the pharmacokinetics of levamisole after oral administration

Parameter	Parameter value	Standard error
Ka (h^{-1}) (CV = 0)[a]	3.37	2.11
Ka (h^{-1}) (CV = 1)[a]	1.41	0.105
t_0 (h) (CV = 0)[a]	0.636	0.065
t_0, h. (CV = 1)[a]	0.801	0.0936
$Cl_{L,NR}/F$ (l h^{-1})[b]	27.7	4.73
V_L/F (l)	207	27.6
$Cl_{L,R}$ (l h^{-1})	3.60	0.641
$Cl_{OH,R}$ (l h^{-1})	11.1	1.26
θ_{10}[c]	0.855	0.0798

[a] CV = 0, 1 indicates the absence, presence of cardiovascular complications

[b] Determined by multiplying the weight normalized $Cl_{L,M}$ by the average patient weight (78.8 kg) and adding to $Cl_{L,OH}$ to give total, non-renal levamisole clearance

[c] θ_{10} is a measure of the effect of gastrointestinal complications on the fraction of levamisole available after oral administration. See text for explanation

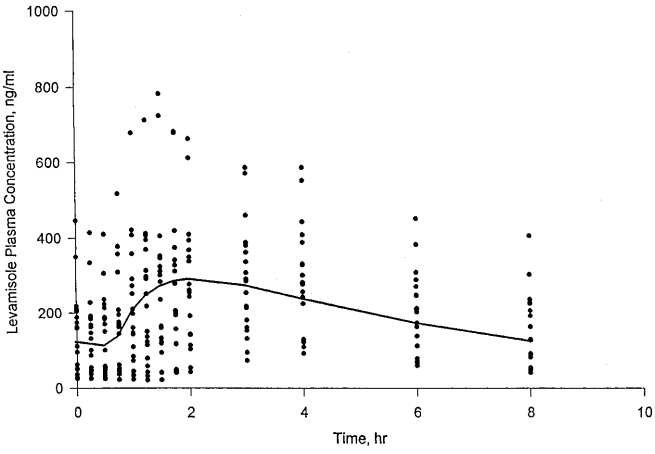

Fig. 1 Observed levamisole plasma concentrations. The solid line is a simulated curve based on the population mean parameter values determined by NONMEM

The subroutine ADVAN5/TRANS1 of NONMEM was used for these estimations. Interpatient variance in the pharmacokinetic parameters was modeled with exponential error. The exponential error model proved superior to both additive and proportional and mixed additive and proportional error models. The residual or intrapatient error which includes error due to model misspecification and assay variability was found to be best modeled using proportional error.

The data were analyzed on the basis that levamisole and *p*-hydroxylevamisole plasma concentrations were at steady-state. This was confirmed by a comparison of the concentrations measured at the beginning and end of the dosing interval using the Wilcoxon signed-rank test.

The effects of various patient covariates on the pharmacokinetic parameters, (specifically weight, age, gender as well as the presence of cardiovascular, gastrointestinal, genitourinal and musculoskeletal disease) were examined. This was accomplished by using the POSTHOC option in NONMEM to generate Bayesian individual parameter estimates and by examining continuous or categorical plots of the estimates versus the various covariates using S-Plus [2]. Evaluation of continuous relationships was aided by fitting data to a general additive model (gam) using the same software.

The initial model developed without covariates produced a minimum value of the objective function (MVOF) which was used as a base value with which to compare the effect of incorporating covariates into the analysis. Covariates were retained which reduced the MVOF by at least four units (corresponding to a *P*-value of <0.05). The resulting intermediate model which included all such covariates was then further refined by removing covariates in a stepwise fashion. To establish the final model, only those covariates were retained which upon removal resulted in an increase in the MVOF of at least 7.88 units (corresponding to a *P*-value of <0.005). To avoid achieving a local rather than a global minimum sum of squares, the initial estimates of the principal parameters determining the pharmacokinetics of levamisole (i.e. absorption rate constant, volume of distribution and clearance to metabolites other than *p*-hydroxylevamisole) were changed by 10%. In all cases the respective parameters achieved identical final values.

Results

Large interpatient variability in levamisole plasma concentrations among patients taking identical doses of levamisole was observed (Fig. 1). There was a sevenfold difference between the highest area under the levamisole plasma concentration time curve (4230 ng · h/ml) and the lowest (608 ng · h/ml).

The proposed pharmacokinetic model fitted the data in a reasonably unbiased manner. This was assessed by determining the median prediction error with 95% confidence intervals, 80.6 (−79.1, 240) [17]. The mean pharmacokinetic parameter values for levamisole are shown in Table 1. The values for volume of distribution and clearance are defined relative to F, the fraction of levamisole available to the systemic circulation following oral administration. A significant improvement in the fit was obtained by including an absorption lag time (Fig. 1).

Covariates that were found to significantly affect the pharmacokinetics of levamisole were patient weight, and the existence of concurrent cardiovascular and gastrointestinal disease. The non-renal clearance of levamisole was found to be proportional to patient weight. Both the value of the lag time and the absorption rate constant were influenced by cardiovascular disease as shown in the following relationships:

$$\mathrm{Ka} = \theta_1 \cdot (1 - \mathrm{CV}) + \theta_{12} \cdot \mathrm{CV}$$

$$\mathrm{ALAG1} = \theta_4 \cdot (1 - \mathrm{CV}) + \theta_{11} \cdot \mathrm{CV}$$

where Ka is the absorption rate constant and ALAG1 the absorption lag-time. The parameter CV indicates the presence (CV = 1) or absence (CV = 0) of cardiovascular disease and the θs are the corresponding values of Ka and ALAG1 under those conditions. In addition, the presence of gastrointestinal disease had a significant effect on the parameter F, the fraction of the drug available to the systemic circulation following oral administration as expressed by

$$\mathrm{F} = \mathrm{GI} + (1 - \mathrm{GI}) \cdot \theta_{10}$$

While the specific value of F is not known, the model indicates that the fraction of the oral dose available was statistically significantly different in patients with gastrointestinal disorders. Since the presence of gastroin-

testinal disorder was coded as 1 and apparent normal gastrointestinal function as 0, using the value of θ_{10} of 0.855 (Table 1) it can be calculated that gastrointestinal disorder resulted in an average increase in the fraction of oral levamisole absorbed of 17% [(1 − 0.855)/0.855].

The overall precision of the fit was assessed by determining the median absolute error with 95% confidence intervals, 2000 (1456, 2425) [17]. The precision with which the parameters were estimated, expressed as the coefficient of variation, can be determined by dividing the standard error of the parameter estimate by the corresponding mean value (Table 1) and expressing the result as a percentage [4]. This yields coefficient of variation values of 62.6% (Ka), 10.1% (lag-time), 13.3% (V/F), 17.1% (Cl_{NR}/F), and 17.8% (CL_R). The population variability in the parameters can be determined by taking the square root of each parameter covariance value, ω^2, and similarly expressing the value as a percentage [5]. Coefficient of variation values of 211% (Ka), 2760% (V/F), 59.5% ($Cl_{L,M}/F$), 11.4% ($Cl_{OH,R}$) and 68.9% (CL_R) were determined.

In a similar manner, intrapatient variability which would include assay error, sampling error and model misspecification can be expressed as a percentage by taking the square root of the variance of the residual error, σ^2_{plasma} and multiplying by 100. Thus intrasubject variability for levamisole plasma concentrations was determined to be 22.3%, supporting the suitability of the pharmacokinetic model.

Discussion

A significant finding of this study is the marked intersubject variability in levamisole plasma concentrations following oral administration to patients with colorectal cancer receiving 5-FU adjuvant chemotherapy. This variability is seen in Fig. 1 and in the population variability in the parameters described in the Results section. Luyckx et al. [9] made reference to similar interindividual variability. They suggested that among subjects receiving the same dose, individual levamisole plasma concentrations differentiated responders from nonresponders with respect to the immunomodulating effects of the drug. Whether plasma levamisole concentrations correlate better with response to combined 5-FU/levamisole adjuvant chemotherapy than levamisole dose has yet to be established.

The levamisole pharmacokinetic parameters reported compare with those appearing in the literature. In a recent study, Reid et al. [16] determined descriptive pharmacokinetic parameters (e.g. c_{max}, t_{max}) in patients receiving 5-FU and levamisole concurrently. Their mean value for t_{max} was 2.1 h and c_{max}, adjusting for dose, was 354 ng/ml compared with 2.82 h and 368 ng/ml in the present study. Kouassi et al. [6] have reported a volume of distribution of 266 ± 120 l, total clearance (renal plus non-renal) of 33.8 ± 9.0 l/h and a renal clearance of 1.75 ± 1.71 l/h in healthy subjects and Reid et al. [16] have determined levamisole clearance to be 351 ml/min per m^2, equivalent to 31.3 l/h assuming a standard body surface area of 1.73 m^2. These results are very similar to those presented in this report. Luyckx et al. [9] have reported a volume of distribution of 110 ± 10.2 l and a total clearance of 17.8 ± 1.9 l/h in both healthy subjects and cancer patients. There is therefore no reason at present to believe that 5-FU has a significant effect on the pharmacokinetics of levamisole.

Some progress has been made in this study to account for the large interpatient variability observed among patients receiving levamisole. The non-renal clearance of levamisole was determined to be proportional to total body weight. Gender was specifically tested in the model as others have reported effects of this variable on levamisole pharmacokinetics [6, 9]. However no reduction in the minimum value of the objective function occurred upon inclusion of this covariate in connection with any of the pharmacokinetic parameters of the model. Examination of the medication profiles likewise yielded no obvious influence on intersubject variability. Two concurrent disease states exerted statistically significant effects on the oral input of levamisole. Patients with cardiovascular disease demonstrated an average 26% increase in the absorption lag-time plus a 58% decrease in the apparent absorption rate constant of levamisole. Cardiovascular disease in these patients was characterized primarily by coronary artery disease and mild hypertension. Circulatory failure can lead to increased sympathetic and decreased parasympathetic activity which may reduce gastrointestinal motility and delay gastric emptying. Circulatory failure will also decrease blood flow to the splanchnic region thereby providing an additional mechanism for a reduced rate of drug uptake. Other drugs have similarly shown reduced absorption rates in patients with cardiovascular disease, e.g. digoxin, procainamide, quinidine, and mexiletine [11].

In addition to cardiac disease, aside from the obvious pathophysiological changes caused by colorectal cancer, some patients also experienced constipation or diarrhea and other gastrointestinal complications. An average 17% increase in the availability of levamisole was associated with patients exhibiting concurrent gastrointestinal disorders. Possible mechanisms mediating this increase include decreased gastrointestinal motility, increased permeability of the gut wall and altered intestinal metabolism [18].

Drugs subject to high first-pass effects can demonstrate large intersubject variability in plasma concentrations upon administration of an oral dose. As others have done [6, 9], the fraction of a levamisole oral dose available to the systemic circulation can be estimated assuming (1) complete absorption and (2) elimination solely by the liver. Thus, given these assumptions, approximately 30% of a levamisole oral dose will be eliminated by the liver on first-pass through the liver. This is not likely to contribute to wide fluctuations in levamisole plasma concentrations.

In conclusion, there does not appear to be a significant difference between the pharmacokinetics of levamisole measured in healthy subjects and those in patients with colorectal cancer treated with 5-FU. All levamisole pharmacokinetic studies to date indicate substantial intersubject variability in levamisole plasma concentrations. The source of this variability is at present poorly defined although it is now apparent that patient weight along with cardiovascular and gastrointestinal complications can influence the pharmacokinetics of levamisole. The contribution of interpatient pharmacokinetic variability to differences in clinical outcome among patients is of interest with regard to possible dose individualization and optimization of 5-FU/levamisole adjuvant chemotherapy in patients with colorectal cancer.

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