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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF N-(2-METHYL-2-PROPYL)-3-OXO-4-AZA-5α-ANDROST-1-ENE-17β-CARBOXAMIDE, A 4-AZASTEROID, IN HUMAN PLASMA FROM A PHASE I STUDY

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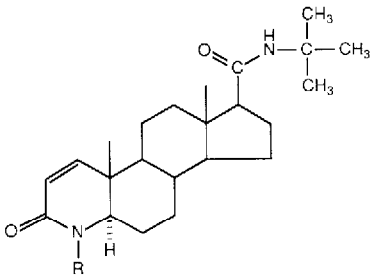
SUMMARY

A sensitive and selective high-performance liquid chromatographic method has been developed for the quantitative determination of N-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (I) in human plasma. I, a 5α-reductase inhibitor and a potential therapeutic agent for benign prostatic hyperplasia, is a member of the family of compounds referred to as the 4-azasteroids. The 4-N-methyl analogue of the drug was used as the internal standard and calibration curves were developed at two levels of sensitivity to cover a large dynamic range of plasma concentrations. Drug was isolated from biological fluids with a solid-phase C_{18} extraction column; the analyte was further purified by adsorption and desorption from a second extraction column (CN cartridge). Evaluation of the isolation method revealed that it was reproducible and drug recoveries equalled ca. 90%. Chromatography was carried out on a C_8 column (5 μm) with ultraviolet detection at 210 nm. The detection limit was ca. 10 ng/ml for I. Human plasma levels are reported for I following single-dose oral administration of 50, 200 and 400 mg of drug; urinary excretion data are reported for a single volunteer given 400 mg of I.

INTRODUCTION

N-(2-Methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (MK-906, I, Fig. 1), a member of the 4-azasteroid family of compounds, is currently undergoing clinical evaluation as an in vivo 5α-reductase inhibitor. The 4-azasteroids are a newly developed family of compounds that block the intracellular metabolism of testosterone (T) to the more potent androgen dihydrotestosterone (DHT) [1–3]. Androgen-sensitive tissues such as the prostate are stimulated primarily by DHT rather than by T, and elevated levels of DHT are related to the pathogenesis of benign prostatic hyperplasia (BPH) [4]. BPH



MK-906 R = H

INTERNAL STANDARD R = CH_3

Fig. 1. Structures of I (MK-906) and the 4-N-methyl analogue of I, the internal standard.

occurs in the majority of men older than 60 years of age and accounts for 250 000 operations yearly [5]. The development of a non-surgical treatment would be of great value [6]. In vivo study of the 4-azasteroids has shown that therapy with these inhibitors reduces prostate growth in rats [7] and dogs [8, 9]. Presumably, lowering the intra-prostatic levels of DHT by selective inhibition of the enzyme 5α-reductase (EC 1.3.1.22) would lead to the regression of prostate growth [10].

High-performance liquid chromatography (HPLC) seems to be a method well suited for the determination of I in biological samples; however, its ultraviolet (UV) absorption is of low intensity and optimal detection is accomplished by monitoring at 210 nm. The drug does not possess any structural feature that could be readily chemically modified to form a derivative with enhanced detectability. As numerous endogenous plasma (and urine) components could be expected to absorb at 210 nm, a selective isolation procedure, based on coupled liquid–solid extractions, was developed to generate an analyte suitable for a sensitive HPLC-based assay for I. Solid-phase extractions have become a well established purification technique. Excellent recoveries, as well as high-quality analytes, have been reported for a wide variety of hormones [11–13] and drugs [14–16] from plasma and urine.

Prior to pharmacokinetic studies of I in human volunteers, a sensitive method for this 4-azasteroid was needed for biological monitoring in a Phase I study. This paper describes an HPLC-based assay for I and its application to the quantification of drug concentrations in the plasma and urine of volunteers given single rising doses of this drug. The methodology, with some modification, is suitable for examining plasma and urine for metabolites of I.

EXPERIMENTAL

Reagents and materials

I and the internal standard (4-N-methyl analogue of I) were synthesized and supplied by Merck Sharp & Dohme Research Labs. (Rahway, NJ, U.S.A.). The

chemical structures of these compounds are given in Fig. 1. Acetonitrile and methanol were purchased from EM Science (Cherry Hill, NJ, U.S.A.); dichloromethane and water were obtained from Mallinckrodt (Paris, KY, U.S.A.). All solvents employed were of HPLC grade and were used without further purification.

Disposable solid-phase extraction cartriges (Sep-Pak) were purchased from Waters Assoc. (Milford, MA, U.S.A.). The Sep-Pak C_{18} cartridges were preconditioned by sequential rinsing with 10 ml of methanol and 15 ml of water. The Sep-Pak CN (cyano propyl) cartridges were preconditioned by sequential rinsing with 10 ml of dichloromethane, 5 ml of methanol and 15 ml of water.

All glassware was washed with detergent, rinsed thoroughly with doubly distilled water and methanol, then dried prior to use.

Chromatographic equipment and conditions

The liquid chromatograph (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) consisted of a Constametric I pump, a Constametric II G pump, and external control gradient master and a Spectromonitor III variable-wavelength UV detector. Injection was performed with a Rheodyne Model 7120 valve fitted with a 1.0-ml loop. The column was a Zorbax C_8 column, 5 μm, 250 mm $\times$ 4.5 mm I.D. (Dupont, Wilmington, DE, U.S.A.). The analytical column was protected by a laboratory-made guard column dry-packed with LC-8 pellicular packing (40 μm, 50 mm $\times$ 4.6 mm I.D.) from Supelco (Bellefonte, P.A, U.S.A.). The chromatograms were recorded on a Model 901-05 linear single-pen strip chart recorder (Altex Scientific, Berkeley, CA, U.S.A.) with an input voltage of 10 mV and a chart speed of 20 cm/h.

The mobile phase composition for the determination of I and the internal standard was acetonitrile–methanol–water (26:39:35) degassed by purging with helium. Chromatography was performed at ambient temperature with a flow-rate of 0.9–1.0 ml/min. The column pressure was 103 bar. The UV detector was set at 210 nm and the sensitivity of the detector was set at either 0.1 absorbance units full scale (a.u.f.s.) for analyses of I and its internal standard in the 0.25–1.5 μg range or 0.02 a.u.f.s. (25–250 ng range).

Human study protocol and sampling handling

Ten healthy male volunteers (age, 18–45 years; body weight, 60–90 kg) participated in this Phase I initial dose-ranging study designed to investigate the tolerability of single rising doses of I. The study was performed at the University of Leuven (Leuven, Belgium) under an institutionally approved protocol. All volunteers gave informed consent. The ten subjects (divided into two panels of five) received three oral doses of I ranging between 12.5 and 400 mg. The interval between active treatments given to the same subjects was one week. Those subjects in panel A were given 12.5, 50 and 200 mg of drug; those in panel B received doses of 25, 100 and 400 mg. All subjects were also dosed with placebo at each dose level, thus, each received a total of six treatments. The subjects, having fasted overnight, were given the medication with a large glass of water (250 ml). At 2 h after taking I they were given a light snack and 4 h post-dose they were given a light lunch.

Blood was drawn into heparinized tubes pre-dose, 4 and 24 h post-dose from subjects given 12.5, 25, 50 and 100 mg of drug. After the 200- and 400-mg doses, blood was taken at the following recorded time intervals: predose, 1, 2, 4, 6, 9 and 24 h. Plasma was separated by centrifugation at 3800 *g* for 15 min and stored frozen at −20°C until analyzed.

Urine specimens were collected pre-dose, 0–6 and 6–24 h after drug administration.

Preparation of stock solutions and standards

Stock standard solutions were prepared for I and its internal standard at 1 mg/ml in methanol. A working solution containing 10 μg/ml was obtained by dilution of the stock solution in methanol.

A stock solution of the standard referred to as the I–internal standard quantitation mixture (1:1) was prepared by combining 0.2-ml aliquots of each of the two stock solutions of I and internal standard and diluting with methanol to a final volume of 10 ml; the quantitation mixture contained 20 μg/ml of both I and internal standard. A working solution for HPLC was prepared from the quantitation standard. The solution, containing 1 μg each of I and internal standard per 25 μl of methanol, was injected into the HPLC system.

Stability data showed that solutions of I and the internal standard were stable in methanol for at least three months at −20°C.

Isolation procedure: plasma

Frozen (−20°C) plasma was thawed at room temperature and then centrifuged at 1500 *g* for 5 min to remove protein precipitates. Depending on the anticipated concentration of I in the subject plasma, either 100 μl (1.0 μg) or 25 μl (250 ng) of the internal standard solution were placed in a centrifuge tube (Pyrex, 15 ml) and the solvent evaporated to dryness under nitrogen. A measured volume (0.4–2 ml) of plasma was introduced into the tube and diluted to 15 ml with water; the tube was stoppered and mixed well. The diluted plasma sample was decanted into the funnel attached to the Sep-Pak cartridge rack (multisample preparation system, Waters Assoc.). Vacuum was adjusted so that the sample and all washes flowed through the preconditioned C_{18} cartridge at 1–2 drops per s. The centrifuge tube was washed with 5 ml of water and this rinse passed through the extraction column. The C_{18} cartridge was washed with 5 ml of methanol–water (50:50, v/v). The vacuum was released and a 100×13 mm collection tube was placed under the cartridge. The C_{18} cartridge was washed with 5 ml of methanol–water (70:30, v/v) to elute a fraction containing I and the internal standard. The tube containing the eluate was placed in a water bath (<50°C) and the eluate was reduced in volume under nitrogen to approximately 1.5 ml in order to decrease the amount of methanol in the isolate. Water (5 ml) was added to the remaining eluate and the resulting solution decanted into the funnel attached to the multisample extraction apparatus. Vacuum was adjusted so that the sample flowed through a preconditioned CN cartridge at 1–2 drops per s; the cartridge remained under vacuum until partially dry. The vacuum was released, a collection tube was positioned under the cartridge which was eluted with about

5 ml of dichloromethane. The eluate was placed in a water bath ($<50°C$) and was evaporated to dryness under nitrogen. The residue was dissolved in 200 μl of methanol with rinsing of the sides of the tube to maximize recovery.

The contents of the collection tube were transferred to a small sample vial. The rinsing of the collection tube with solvent was repeated to ensure good recovery of the analyte. The sample was evaporated (water bath) to dryness under nitrogen. The analyte was dissolved in 25 or 50 μl of methanol and 25 μl were injected into the chromatograph.

The quantitation standard (1:1 mixture of I and its internal standard) in control plasma was prepared by adding either 100 or 25 μl of the quantitation standard solution (2 μg or 500 ng, respectively) to a 15-ml centrifuge tube and removing the solvent. Control plasma (1 ml) was added to the tube, and water added to a total volume of 15 ml. The isolation procedure was then initiated.

Calculations

The peak heights for the UV-absorbing compounds eluted at 13–14 min (I) and 20.5–21.5 min (the internal standard) were measured in mm. The I/internal standard peak-height ratio was calculated for the quantitation standard in the volunteer's drug-free plasma. The concentration of I in the volunteer's drug-treated plasma was determined by comparing directly the resulting peak-height ratio with that of the appropriate level quantitation standard (250 ng or 1.0 μg).

Isolation procedure: urine

A 100-μl (1.0 μg) volume of the internal standard solution was placed in a 50-ml centrifuge tube; the solvent was evaporated to dryness under nitrogen. A 5-ml volume of urine was pipetted into the tube and diluted to 10 ml with water. The tube was stoppered and vortexed. I and its internal standard were extracted by adding two 10-ml volumes of dichloromethane. The organic layers were separated by centrifugation at 1500 *g* for 10 min, chilled to force out precipitates that were also separated by centrifugation (as above); the supernatant was evaporated to dryness under nitrogen. The dried analyte was redissolved with a 1-ml volume of methanol and diluted to 15 ml with water. The diluted urine extract was adsorbed onto a C_{18} cartridge and from this point processed and calculated as described above for plasma. The amount of I (μg/ml excreted $\times$ total number of ml of urine) recovered from urine was calculated.

Preparation of calibration standards

Calibration standards for generating standard curves at two levels of sensitivity were prepared by combining appropriate volumes of the respective working solutions of I and the internal standard. Aliquots of each working solution, representing either nanogram or microgram amounts of drug, were pipetted into a series of centrifuge tubes (Pyrex, 15 ml). The solvent was evaporated to dryness under nitrogen and a 1.0-ml volume of drug-free plasma was added to each. These tubes contained either 1 μg of internal standard and 0.25, 0.50, 0.75, 1.0 or 1.5 μg of I for the upper range standard curve or 250 ng of internal standard and 25, 50, 100, 175 or 250 ng of I for the lower range curve.

Both sets of calibration standards were processed according to the procedure described above. Calibration curves were constructed by plotting peak-height ratios of I to the internal standard versus the concentrations of I in plasma and fitting the data with a linear regression.

Assay validation

Selectivity of the assay was determined by examining each volunteer's drug-free plasma to confirm the absence of co-eluting peaks.

Accuracy and precision of the method were demonstrated by assaying replicate ($n=5$) samples of drug-free plasma containing I at concentrations of 25, 250 and 1000 ng/ml. The samples were treated as unknows and quantitation was achieved by interpolation of the respective peak-height ratios from the appropriate standard curve.

The reproducibility of the assay was determined by assaying the drug-free plasma from each volunteer spiked with I and the internal standard at a concentration of 1.0 μg/ml (1:1 mix solution). The observed I/internal standard peak-height ratios with all volunteer plasma were compared over the two-month period the data were generated and the coefficient of variation was calculated. Recovery was calculated by comparison of the peak heights of the extracted standards with the peak heights of the unextracted standards (in methanol) which were injected directly on-column.

The minimum quantifiable concentration of drug was established by analyzing drug-free plasma spiked with I at a concentration of 10 ng/ml.

RESULTS AND DISCUSSION

Isolation

In the development of this assay methodology several problems had to be resolved. First, it was necessary to develop an isolation procedure that would provide efficient extraction of I and its internal standard from plasma with minimum interference from the endogenous UV-absorbing components that would be detected at 210 nm. The lack of a useful chromophore or derivatizable functional group makes I a difficult compound to assay with optimum sensitivity and specificity. Second, chromatographic conditions had to be established to obtain baseline separation of I, its metabolites and the internal standard. Lastly, since this study represented the initial clinical study in man, plasma concentrations of I were unknown and unpredictable, and thus the assay needed to possess a large dynamic range of sensitivity and flexibility.

The method for the isolation of I from human plasma employs two solid-phase extraction cartridges and was carried out according to the procedure shown in Fig. 2. One of the principal advantages of liquid–solid extractions is that, unlike liquid–liquid extractions, by choosing suitable selective adsorbents the partition equilibrium of specific sample components can be driven to favor nearly complete adsorption or desorption. The combination of the non-polar C_{18} cartridge and relatively more polar CN cartridge gave good separation between plasma matrix constituents (proteins, lipids and organic acids), I and related species. Selective

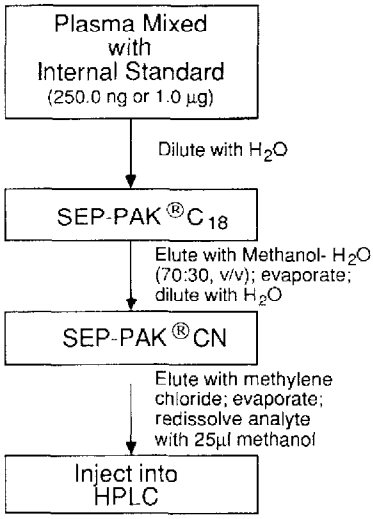


Fig. 2. Procedure for the isolation of I from plasma.

washing and elution of the C_{18} solid-phase extraction column eliminated much of the early-eluting polar UV-adsorbing material that would normally obliterate the first 6–9 min of the chromatogram. In contrast, elution of the CN cartridge with dichloromethane reduced the occurrence of late-eluting non-polar UV-absorbing components that contribute to ghosting. Extraction of the drug-containing C_{18} cartridge eluate with dichloromethane was not as effective a purification step as the use of the CN cartridge. At 210 nm, reduction in the late-eluting UV response arising from the physiological matrix minimized the time between injections.

The use of the multisample apparatus designed for solid-phase extraction columns enabled eight isolation procedures to be performed simultaneously. The 4-N-methyl analogue of I served as the internal standard, it met the requirements for quantitative recovery from plasma and, more importantly, the results of earlier work showed that it was unlikely that the 4-N-methyl compound would arise as a metabolite of I. The chromatographic conditions described in Experimental provided excellent resolution for I in plasma; however, speed and sensitivity of analysis for drug are less than optimal. The operating conditions of the C_8 column were chosen for the purpose of generating pertinent metabolism data as well as quantifying drug. Monohydroxylated metabolites of I, identified earlier in the rat and the dog [17], were significantly more polar than parent drug.

Recovery

As compared to the peak-height intensities exhibited at 210 nm by reference samples spiked into drug-free plasma, the absolute recoveries of I and its monohydroxymethyl metabolite taken through the isolation procedure were determined to be 90 and 70%, respectively. These UV-based data were confirmed by the use of radiolabeled compounds. A comparison of the peak-height responses

measured for I and its interal standard during the course of this investigation indicated that assay recoveries were highly consistent at both ranges of sensitivity.

Chromatography

Under the chromatographic conditions employed (described in Experimental) I and the internal standard were well separated from each other and possessed retention times of 13 and 21 min, respectively. The total run time equalled 30 min.

Calibration curves and detection

It was anticipated that plasma from this initial Phase I study could contain widely different concentrations of drug; therefore, prior to the arrival of the samples, calibration curves were developed at two levels of sensitivity for I (10–250 ng/ml and 0.1–1.5 μg/ml). The slope, intercept and correlation coefficients were determined by linear regression analysis of the peak-height ratios of I/internal standard versus the known concentrations of drug in the analyte. The calibration data for both curves (nanogram and microgram ranges) were well described by a linear equation ($y=mx+B$) with correlation coefficients of 0.9995 and 0.9999, respectively.

Based on a signal-to-noise ratio of 2:1 the limit of detection for I was 10 ng/ml. The detection limit, however, could be lowered if a larger aliquot of plasma was assayed. The isolation procedure can easily handle larger volumes of plasma (up to 3 ml) without significantly increasing the UV response from endogenous components. When used in trace analysis a solid-phase cartridge acts as a concentration device, and thus the absolute amount of drug in the initial sample is more important than the actual plasma volume taken for assay. In the present investigation, the capacity of the C_{18} cartridge to adsorb microgram amounts of drug was not exceeded.

Assay accuracy, precision and reproducibility

The accuracy of the assay was expressed as: the mean observed concentration/expected concentration (25, 250 or 1000 ng/ml) $\times 100$. The results presented in Table I indicated that, in the worst case, any single analysis would fall within 88% of its true value. The precision (within-day variability) is also dem-

TABLE I

VALIDATION OF THE METHOD

Assay accuracy and precision for I in human plasma by use of spiked samples ($n=5$).

Nominal concentration (ng/ml)	Obtained concentration (ng/ml)	Error* (%)	Coefficient of variation (%)
25	22	12	3.4
250	230	8	3.4
1000	973	3	4.5

*95% Confidence limit of true value.

onstrated by the data in Table I. The coefficient of variation (C.V.) averaged 3.8% over the range 25–1000 ng/ml I. Samples ($n=5$) spiked at a concentration of 1.0 μg/ml drug showed a mean value of 0.95 ± 0.02 μg/ml which gives a C.V. of 4.5%. The reproducibility (day-to-day variability) of the assay as determined over a two-month period was 3.4% at 1.0 μg/ml.

Application of the method

The assay was used to quantify I in plasma from healthy volunteers given both placebo and single rising oral doses of drug. Representative examples of the chromatograms obtained at 210 nm for drug-free plasma and for drug-free plasma spiked with a 1:1 mixture containing either 250 ng or 1.0 μg of I and the internal standard are shown in Figs. 3A and B and 4A and B. Clearly, at both ranges of detector response (0.02 or 0.1 a.u.f.s.) there is minimal interference; I and the internal standard were resolved from all significant UV-absorbing endogenous plasma components and from potential metabolites.

The assay was first employed to examine plasma from volunteers given both placebo and single 50-mg doses of I. Because lower amounts of drug were anticipated, the plasma was mixed with 250 ng of the internal standard, then subjected to the same analysis used to develop the nanogram-level calibration curve. Fig. 3C shows a typical chromatogram obtained with plasma from a volunteer 4 h post-dose. The chromatogram exhibited a dominant UV-absorbing component eluting at the retention time of I (13 min). Two low-intensity peaks not observed in the chromatogram of the drug-free analyte (Fig. 3A) were noted at retention times of 6 and 7 min and thus were recognized as possible metabolites. After the quantitative analyses were completed, the remainder of the 4-h post-dose plasma samples were combined and this pooled plasma served as the source of material for a follow-up structure study. The component, thought to be I, was isolated by HPLC and examined by mass spectrometry; the mass spectrum of the analyte matched that of I, confirming the identity of the observed (and quantified) UV-absorbing component as parent drug. Although not presented in Fig. 3, the analogous chromatogram obtained with the 24-h post-dose plasma from this volunteer showed that drug had depleted to very low levels (<20 ng/ml). Structure confirmation studies could not be undertaken with such a small amount of drug; however, cochromatography of the putative drug peak with 20 ng of reference compound was performed to demonstrate that the plasma peak of low intensity co-eluted with I. Mean plasma concentrations of I from three volunteers given a single 50-mg oral dose are presented in Table II.

Determination of the oral absorption profile of I in volunteers given placebo and either 200 or 400 mg of drug was the major intent of the overall study. Blood samples were taken at 1, 2, 4, 6, 9 and 24 h post-dose. Based on the limited amount of data generated by the previously described study (50-mg dose), plasma samples were assayed for I by use of the standard curves generated at the microgram range. That is, a given volume of plasma (depending on the anticipated drug concentration) was mixed with 1 μg of the internal standard and subjected to the analytical procedure. If an analysis fell outside the upper range of the standard curve an appropriately smaller volume of plasma was reassayed. Fig. 4C shows a

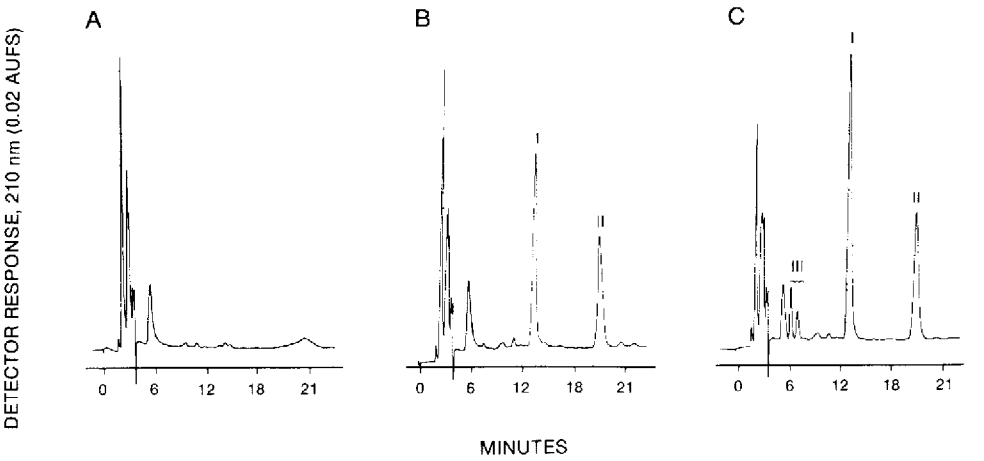


Fig. 3. Representative chromatograms of (A) blank human plasma, (B) blank plasma containing 250 ng/ml I and 250 ng/ml internal standard and (C) plasma collected from a human volunteer 4 h after a single oral 50-mg dose of I. Conditions: column, Zorbax C_8; mobile phase, acetonitrile–methanol–water (26:39:35); flow-rate, 1.0 ml/min. Peaks: I = MK-906; II = internal standard; and III = metabolites.

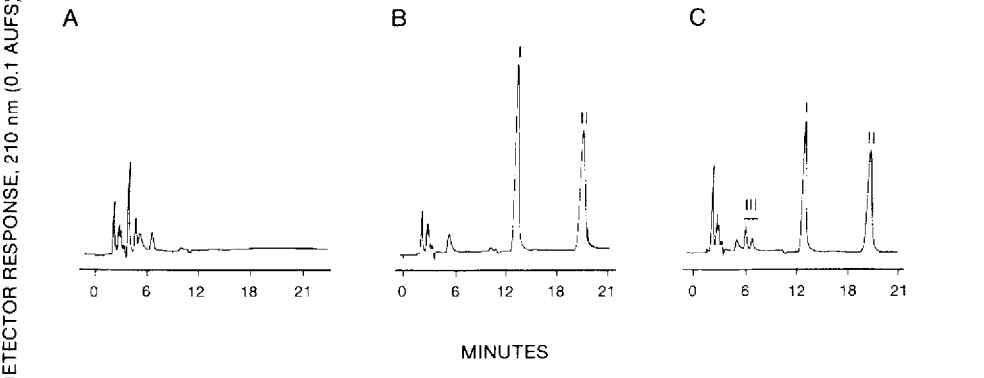


Fig. 4. Representative chromatograms of (A) blank human plasma, (B) blank plasma containing 1.0 μg/ml I and 1.0 μg/ml internal standard and (C) plasma collected from a human volunteer 4 h after a single oral 400-mg dose of I. Conditions and peaks in Fig. 3.

TABLE II

CONCENTRATIONS OF I IN PLASMA FROM HEALTHY VOLUNTEERS FOLLOWING SINGLE ORAL DOSES OF DRUG

Values indicate mean ± S.E. for the number of determinations shown in the second column. After the 50-mg dose blood was taken 4 and 24 h post-dose.

Dose (mg)	n	t_{max} (h)	C_{max} (μg/ml)	$C_{24\,h}$ (μg/ml)	$t_{1/2}$ (h)	$AUC_{0-24\,h}$ (μg·h/ml)
50	3	4	0.29 ± 0.02	0.015 ± 0.007	–	–
200	5	2–4	0.92 ± 0.08*	0.29 ± 0.06	17.3 ± 3.5	12.9 ± 1.12
400	5	4–6	2.05 ± 0.19*	0.72 ± 0.02	13.4 ± 0.73	31.5 ± 1.67

*Values indicate mean ± S.E. for ten data points.

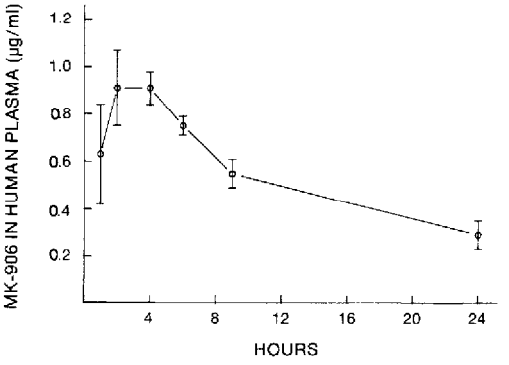


Fig. 5. Mean plasma levels of I from human volunteers ($n=5$) after oral intake of 200 mg of drug.

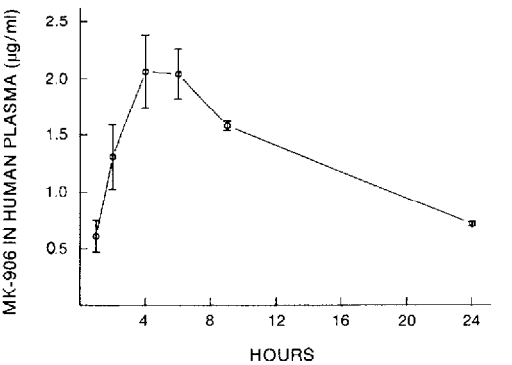


Fig. 6. Mean plasma levels of I from human volunteers ($n=5$) after oral intake of 400 mg of drug.

typical chromatogram obtained with plasma from a volunteer 4 h after a 400-mg dose of I. Remarkably, all the UV chromatograms arising from the HPLC analysis of plasma from the volunteers given 200 and 400 mg of drug exhibited qualitatively similar profiles with regard to parent drug and metabolites. The resulting chromatograms possessed a major UV-absorbing peak at the retention time of I and very-low-intensity metabolite peaks (labeled as III on the chromatogram) eluting between 6 and 7 min. During the course of each HPLC analysis the putative drug peak was collected; examination of this fraction by mass spectrometry revealed that the UV peak exhibited a mass spectrum in full agreement with that of I. Mean plasma concentrations of I at 4 and 24 h post-dose for five volunteers following single 200- and 400-mg doses of I are presented in Table II; mean plasma concentration–time curves for both doses are presented in Figs. 5 and 6, respectively. The terminal plasma half-life was estimated from the means of the 9- and 24-h data points; the $AUC_{0-24\,h}$ values were calculated.

The pharmacokinetic data are presented in Table II. The half-life values observed following the 400-mg doses exhibited a relatively small amount of intersubject variation and were comparable to those observed most often following the 200-mg dose. A comparison of the mean plasma concentration–time curves, via their respective $AUC_{0-24\,h}$ values, indicated that following 200- and 400-mg single oral doses of I, the response with respect to dose was linear.

The assay in the modified form described in Experimental was used to quantify I in urine (0–6 and 6–24 h) from one of the volunteers given 400 mg of drug. The results of a preliminary investigation revealed that the concentration of I in urine was very low (ca. 150 ng/ml); therefore, 5-ml volumes of urine were taken for analysis. The amount of parent drug excreted in the 0–24 h urine accounted for less than 0.1% of the dose. As conjugates of I itself are unlikely, hydrolysis of urine would not be expected to release any significant amount of parent drug. Urinary profiles, however, were dominated by several polar UV-absorbing peaks which exhibited the same HPLC behavior (retention times of 6–7 min) as the monohydroxylated plasma metabolites. Semi-quantitative estimations of the

amount of each metabolite present in the free neutral fraction of the 0–24 h urine were performed by direct comparison of metabolite peak-height responses at 210 nm with the peak-height response from a known quantity of a reference compound. These data indicated that neutral metabolites accounted for less than 0.5% of the dose. One can speculate that larger amounts of the hydroxylated metabolite of I are excreted as polar conjugates; however, these initial results suggest that elimination via the fecal route is more important after dosing with I.

CONCLUSIONS

The methodology developed in this work for the quantification of I is accurate, precise, reproducible and sufficiently sensitive to determine drug concentration with plasma up to 24 h post-dose. One of the most valuable features of the method is its selectivity, that is, the ability to reduce to very low intensities the UV responses of the endogenous plasma components which are usually significant at 210 nm. Because background interferences were minimal at the retention times of interest, the UV response of the peak for I could be compared with that arising from possible metabolite peaks. Thus the main objectives of our investigation were accomplished. Oral absorption profiles and terminal half-lives for I were established at two doses and human metabolism data were generated as part of a Phase I study wherein no radiolabeled drug was used. Knowledge of plasma levels, depletion rates and linearity of dose response arising from early clinical studies permits rational, and possibly safer, choice of dose level to achieve desired plasma concentrations of a therapeutic agent. The rather high and sustained plasma levels of I combined with its modest conversion to circulating metabolites contribute to making I an attractive drug candidate for use against BPH.

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