
The Pharmacokinetics of 2.5- to 10-mg Oral Doses of Minoxidil in Healthy Volunteers

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The dose proportionality of minoxidil was investigated by studying its pharmacokinetics after administration of single, oral doses of 2.5, 5.0, and 10.0 mg. The study, which was a Latin square cross-over design, was performed in 30 young, nonobese, normal subjects. Treatments were separated by a 4-day washout period. Serum and urine levels of minoxidil were determined by high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA). Supine blood pressure and pulse were monitored during each study phase. Minoxidil concentrations determined by RIA were highly correlated with concentrations determined by HPLC; only the HPLC data was used in the pharmacokinetic analyses. No significant effects were observed for dose normalized C_{max} , t_{max} , volume of distribution, minoxidil renal clearance, or the percentage of the dose excreted as either minoxidil or minoxidil glucuronide. Significant differences in apparent oral clearance, dose normalized AUC, and terminal elimination rate constant (β) were observed between the 2.5-mg dose and the higher doses, but no differences in these parameters between the 5.0- and 10.0-mg doses were apparent. Thus, the available data support dose-independent pharmacokinetics for minoxidil over this range of doses. Repeated measures analysis of variance detected significant time and treatment effects on supine blood pressure and pulse rate, but the effects were generally small and of little clinical significance. The results support the hypothesis that minoxidil has little effect on blood pressure in normotensive subjects.

Minoxidil is a direct acting vasodilator used in treating hypertension. Minoxidil causes relaxation of vascular smooth muscle, resulting in a decrease in peripheral vascular resistance and a drop in blood pressure.¹ Decreases in peripheral resistance and blood pressure lead to baroreceptor reflex stimulation and compensatory increase in heart rate.^{1,2} Minoxidil, in combination with a beta-blocking agent and a diuretic, is indicated for treatment of hypertension refractory to other therapies.¹⁻³ The precise mechanism of minoxidil action is currently unknown.³

Although minoxidil has been on the market for some time, relatively little is known concerning its pharmacokinetics, particularly in normal subjects. Information concerning minoxidil kinetics in dis-

ease states, as well as data concerning the relation of minoxidil pharmacokinetics to dose, is also lacking. In addition, the kinetics of minoxidil usually have been studied using radioimmunoassay (RIA) methodology for sample analysis, whereas a more specific high-performance liquid chromatography (HPLC) method is available currently. Therefore, this study had three objectives: to determine the relationship between minoxidil pharmacokinetics and dose, to determine the relationship between blood pressure lowering and minoxidil dose, and to compare RIA and HPLC methods for determining minoxidil concentrations in serum and urine. These objectives were met by studying the pharmacokinetics of minoxidil in young, healthy, nonobese volunteers after administration of 2.5-, 5.0-, and 10.0-mg oral doses of minoxidil.

METHODS

Subject Selection

The study was approved by the local institutional review board, and each subject provided written in-

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formed consent before participating in the study. Thirty healthy, nonobese volunteers between the ages of 18 and 55 were recruited for the study. For all subjects, results from physical examinations and electrocardiograms were normal. General health screens, including complete blood count with differential and platelets, urinalysis, and clinical chemistry screens, were also normal. Subjects with upper GI diseases, renal or hepatic impairment, cardiovascular disease, or sensitivity to minoxidil were excluded from the study, as were pregnant women. Subjects did not receive any known enzyme-inducing agents for 30 days before the study, and they received no medications for the 7 days preceding the study. Subjects received no drugs, other than those specified in the protocol, throughout the study period. Subjects were permitted to smoke their customary number of cigarettes during the study.

Drug Administration and Sample Collection

The study was a Latin square design with three treatments: treatment A, one 2.5-mg minoxidil tablet (LONITEN® tablets, The Upjohn Co, Kalamazoo, MI) orally; treatment B, two 2.5-mg minoxidil tablets orally; and treatment C, one 10-mg minoxidil tablet orally. Study phases were separated by 4 days. Subjects fasted from 10 PM the evening before the dose until 4 hours after the dose. Standard meals were then consumed at 11 AM, 5 PM, and 10 PM. Minoxidil doses were administered at 7 AM. Venous blood samples (10 mL) were collected just before the dose, at 10, 20, 30, and 40 minutes and at 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 hours after drug administration. Blood samples were collected in nonheparinized vacutainers and allowed to clot. Serum was harvested after centrifugation and frozen at -20°C until analyzed. Urine was collected during the following intervals: 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours. The urine volume collected in each interval was recorded, and two 20-mL aliquots were removed and frozen until subsequently analyzed.

Supine blood pressure and pulse rates were determined just before and at 1.25, 2.25, 3.25, 4.25, 6.25, 8.25, 10.25, and 12.25 hours after minoxidil administration.

Serum and Urine Analysis

Serum was analyzed for minoxidil base by HPLC and RIA. Total (unconjugated and conjugated) minoxidil was determined by RIA after the serum was treated with β -glucuronidase. Total (free and conjugated) and unconjugated minoxidil in urine were

determined by HPLC and by RIA. Urine minoxidil glucuronide concentrations, expressed as minoxidil equivalents, were calculated as the difference between total and unconjugated minoxidil concentrations.

RIA assays in serum and urine were performed by the method of Royer et al.⁴ Minoxidil serum concentrations were also determined by a specific HPLC method.⁵ Briefly, this method consisted of a solid-phase extraction of minoxidil and internal standard (N,N-dipropylminoxidil) from serum. Chromatographic resolution and quantification of the peaks of interest were accomplished using a reversed phase C_{18} column, with the mobile phase (methanol:water:glacial acetic acid:sodium dioctylsulfosuccinate—1500 mL:560 mL:10 mL:3 g) flow rate set at 0.5 mL/minute. Column eluent was monitored at 280 nm using an UV detector. Standard curves were linear from 1–10 ng/mL and 10–300 ng/mL. Within-day coefficients of variation were less than 8.2%; between-day CVs were less than 6.4%.

Urine minoxidil content was determined by a similar procedure. Standard curves were linear from 5 to 50 ng/mL and from 50 to 500 ng/mL. Coefficients of variation for this method were less than 6.8%.

Data Analysis

Pharmacokinetic analyses were performed using the HPLC generated data. The terminal elimination rate constant (β) was determined by simple regression of the linear terminal portion of the log concentration-time profile for minoxidil. The area under the serum minoxidil concentration-time profile (AUC) was calculated up to the last measurable concentration (C_{last}) by trapezoidal rule and extrapolated to infinity by adding C_{last}/β . The apparent oral clearance (CL_o) was calculated as dose/AUC . Apparent volume of distribution (Vd/F)— F = bioavailability—was calculated by dividing CL_o by β . Peak serum minoxidil concentrations (C_{max}) and the times at which they occurred (t_{max}) were determined graphically. The total amounts of minoxidil and minoxidil glucuronide excreted in the urine were calculated by summing the amounts excreted over each interval. The renal clearance of minoxidil (CL_R) was calculated as the amount of minoxidil excreted in the urine divided by serum minoxidil AUC. Differences in the pharmacokinetic parameters among treatments were assessed by analysis of variance using SAS.⁶

Differences in blood pressure and pulse rate among treatments were assessed by repeated measures analysis of variance (ANOVA). If significant time or treatment effects were observed, comparisons of treatment within time and time within treatment were performed.

The RIA and HPLC results were compared by linear regression and correlation analysis.

RESULTS

Twenty-nine subjects completed the study. Subject 16 did not complete phase III for personal reasons; data for this subject from the two completed phases were included in the analysis. Minoxidil serum concentration-time data generated by HPLC were not available for subject 2. Therefore, only data from 29 subjects were used in the analysis of serum minoxidil pharmacodynamics.

Linear regression analyses of the concentration-time data determined by HPLC and RIA analysis of unconjugated minoxidil in serum, unconjugated minoxidil in urine, and total minoxidil in urine, exhibited significant correlations between the two methodologies (r^2 values of .947, .970, and .978, respectively, $P < .0001$ in all cases). The slopes of the respective regression lines of RIA versus HPLC concentrations were 1.08, 0.894, and 1.03.

Mean minoxidil plasma concentration-time profiles are shown in Figure 1. Minoxidil pharmacokinetic parameters are summarized in the Table. Significant treatment effects were noted for AUC and dose-normalized AUC ($P < .0001$ and $P = .0162$, respectively), as well as for C_{max} ($P < .0001$). Significant treatment effects were also noted for Cl_o and Cl_o corrected for body weight ($P = .0008$ and $P = .0022$, respectively). The analysis of these parameters was complicated, however, by significant period effects ($P = .0009$ and $.0008$, respectively). Linear regression analysis showed a significant correlation between minoxidil AUC and dose ($r^2 = .782$, $P < .0001$).

The amounts of minoxidil and minoxidil glucuronide excreted in the urine over 96 hours are presented in the Table. Minoxidil and minoxidil glucuronide in the urine accounted for approximately 20% and 43% of the dose, respectively. No significant treatment effects on the percentage of minoxidil and glucuronide excreted in the urine were observed. The renal clearance of minoxidil was also unaffected by treatment ($P = .0861$).

Figures 2 through 4 depict the mean values for blood pressure and pulse rate measurements as a function of time for the three minoxidil doses. Repeated measures ANOVA of blood pressure and pulse rate revealed significant time and treatment effects on these parameters. Subsequent analysis of blood pressure and pulse rate, evaluating treatment effects at each measurement time and time effects within each treatment, indicated significant differences in pulse rate measurements among treatments only at 2.25 and 3.25 hours. Significant effects of

TABLE I

Mean ($\pm$ SD) Pharmacokinetic Parameters for Minoxidil at Three Dosage Levels in 29 Young Normal Volunteers

	Treatment		
	A	B	C
Dose (mg)	2.5	5.0	10.0
No. of subjects	29	29	28
AUC (ng · hr/mL)	25.3* (7.02)	55.1* (12.1)	112* (30.4)
Dose normalized* AUC (ng · hr/mL)	25.3† (7.02)	27.5 (6.06)	28.0 (7.60)
Cl_o (L/hr)	107† (30.6)	95.2 (21.5)	95.5 (26.0)
Cl_o (L/hr/kg)	1.56† (0.43)	1.41 (0.33)	1.41 (0.38)
C_{max} (ng/mL)	16.8* (7.83)	37.2* (12.1)	74.7* (48.3)
Dose normalized* C_{max} (ng/mL)	16.8 (7.83)	18.6 (6.05)	18.7 (12.1)
t_{max} (hr)	0.566 (0.679)	0.389 (0.107)	0.419 (0.165)
β (hr ⁻¹)	0.646† (0.230)	0.544 (0.332)	0.517 (0.113)
$t_{1/2}$ (hr) ^b	1.07	1.27	1.34
Vd/F (L)	173 (49.1)	184 (62.0)	196 (80.6)
Vd/F (L/kg)	2.53 (0.62)	2.67 (0.62)	2.81 (0.88)
CL_R (L/hr)	21.1 (5.19)	19.3 (5.28)	18.6 (5.73)
Amount of minoxidil in urine (fraction of dose)	0.196 (0.059)	0.198 (0.050)	0.206 (0.057)
Amount of MG ^c in urine (fraction of dose)	0.443 (0.118)	0.424 (0.111)	0.422 (0.095)

* Treatments A, B, and C are significantly different from one another at $P < .05$ by Duncan's multiple range test.

† Significantly different from treatments B and C at $P < .05$ by Duncan's multiple range test.

^a Normalized to a 2.5-mg dose.

^b Harmonic mean.

^c MG = minoxidil glucuronide.

Cl_o = oral clearance.

time within treatment were noted for all parameters within all treatments except systolic blood pressure measured in subjects in treatment group A.

DISCUSSION

Typically, minoxidil pharmacokinetics have been evaluated using RIA.⁶ RIA methodologies are sensitive but suffer from the possible interaction of metabolites with the antibody, resulting in erroneously high values for parent drug concentrations. In this

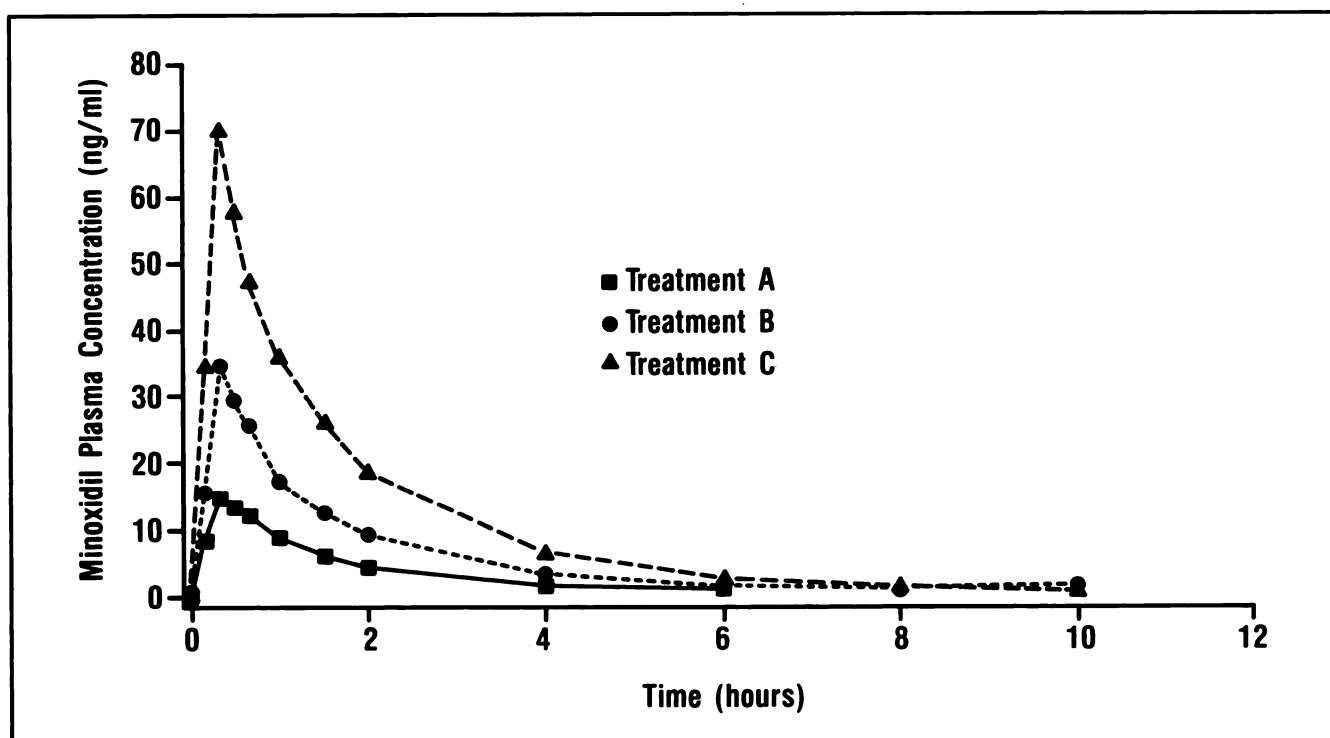


Figure 1. Mean minoxidil serum concentrations after single oral doses of 2.5 mg (treatment A), 5.0 mg (treatment B), and 10.0 mg (treatment C) in 30 normal volunteers.

experiment, a specific HPLC assay was compared with RIA assay results. Serum minoxidil free base and urine total (conjugated and unconjugated) concentrations determined by RIA were consistently higher than those determined by HPLC, suggesting possible metabolite interference. However, urine minoxidil free base concentrations determined by RIA were lower than corresponding HPLC values. The pharmacokinetic data analyses in this report were performed using concentration-time data determined by HPLC; similar results and conclusions also resulted from the analysis of the RIA data.

The pharmacokinetic data were generally supportive of dose-independent pharmacokinetics over the range of doses studied. There were, however, treatment effects on Cl_o , β , and dose-normalized AUC. Significant differences in these parameters were observed between the 2.5-mg dose and the 5.0-mg and 10-mg doses; there was no difference in these parameters between the higher doses. Because of the limited sensitivity of the assay and the low concentrations observed after the 2.5-mg dose, the differences in Cl_o , β and dose-normalized AUC were probably due to having insufficient data for pharmacokinetic analysis rather than concentra-

tion-dependent pharmacokinetics. The lack of difference in these parameters between the 5-mg and 10-mg doses, as well as the linear relationship between AUC and dose more strongly support dose-independent pharmacokinetics over this range of doses.

The urinary excretion data was also supportive of dose-independent pharmacokinetics. No significant treatment effects on CL_R , or the percentage of the dose excreted as minoxidil and minoxidil glucuronide were observed. The percentage of the dose excreted as minoxidil (20%) was somewhat higher than the 12% previously reported.¹ This observation may be due to the fact that hypertensive subjects with some degree of renal dysfunction were used in earlier pharmacokinetic studies^{7,8}; the renal clearance of minoxidil would thus be higher in normal subjects. The fraction of the dose excreted as minoxidil glucuronide agreed well with the previously reported value of 38.5%.⁸

Repeated measures ANOVA revealed borderline significant treatment and time effects ($P = .0779$ and $P = .0203$, respectively) on both systolic and diastolic blood pressure. When systolic and diastolic blood pressure results were analyzed by ANOVA at each

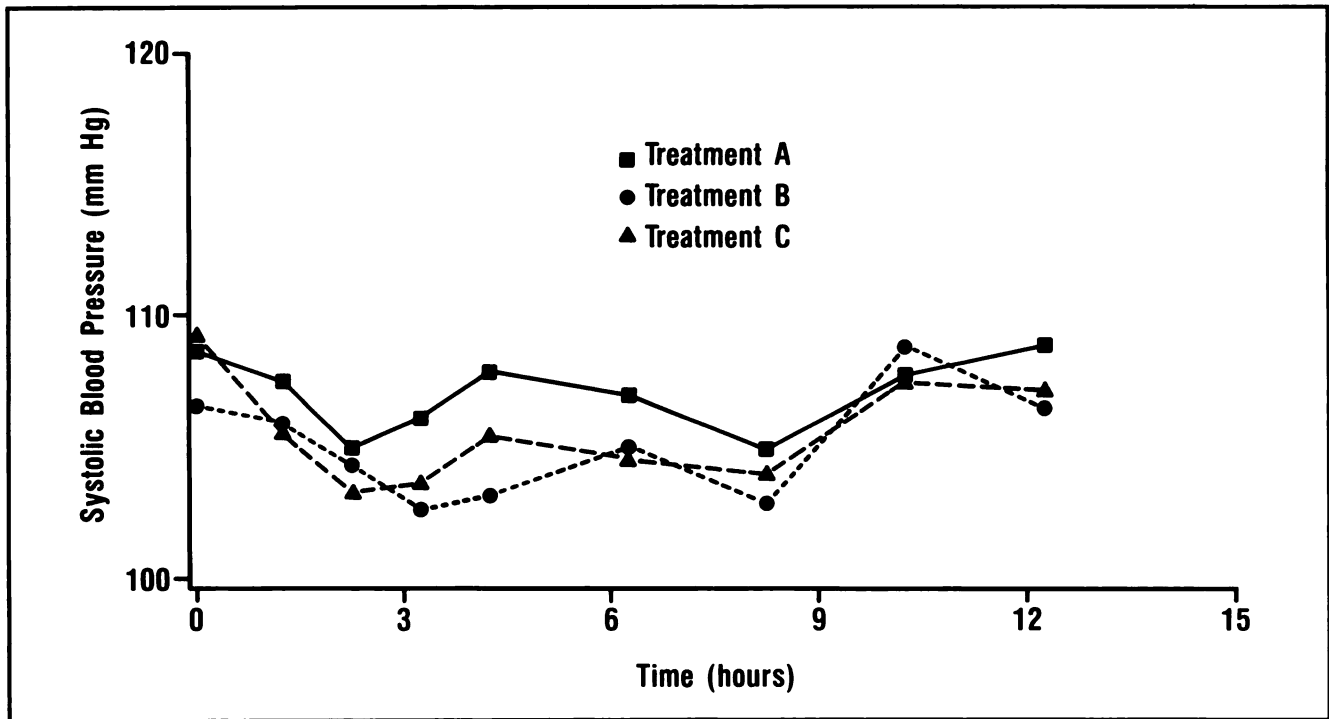


Figure 2. Mean supine systolic blood pressures after single oral doses of 2.5 mg (treatment A), 5.0 mg (treatment B), and 10.0 mg (treatment C) minoxidil in 30 normal volunteers.

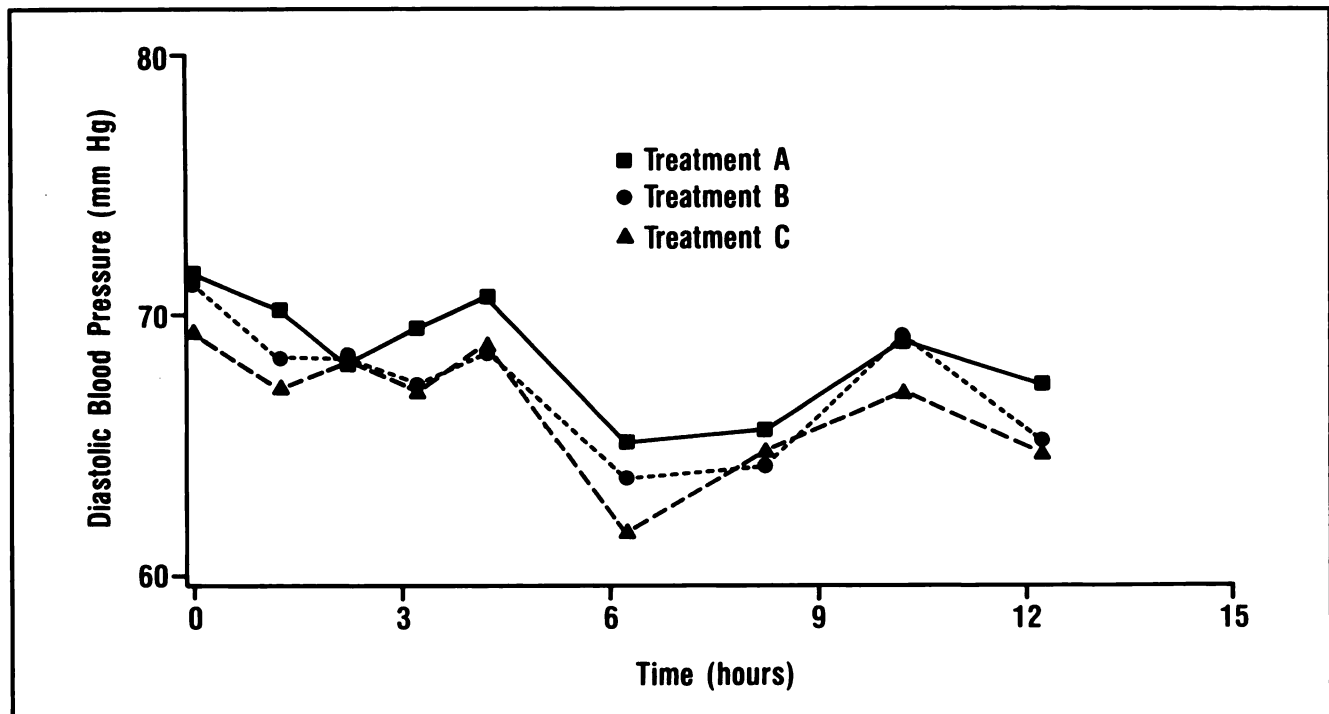


Figure 3. Mean supine diastolic blood pressures after single oral doses of 2.5 mg (treatment A), 5.0 mg (treatment B), and 10.0 mg (treatment C) minoxidil in 30 normal volunteers.

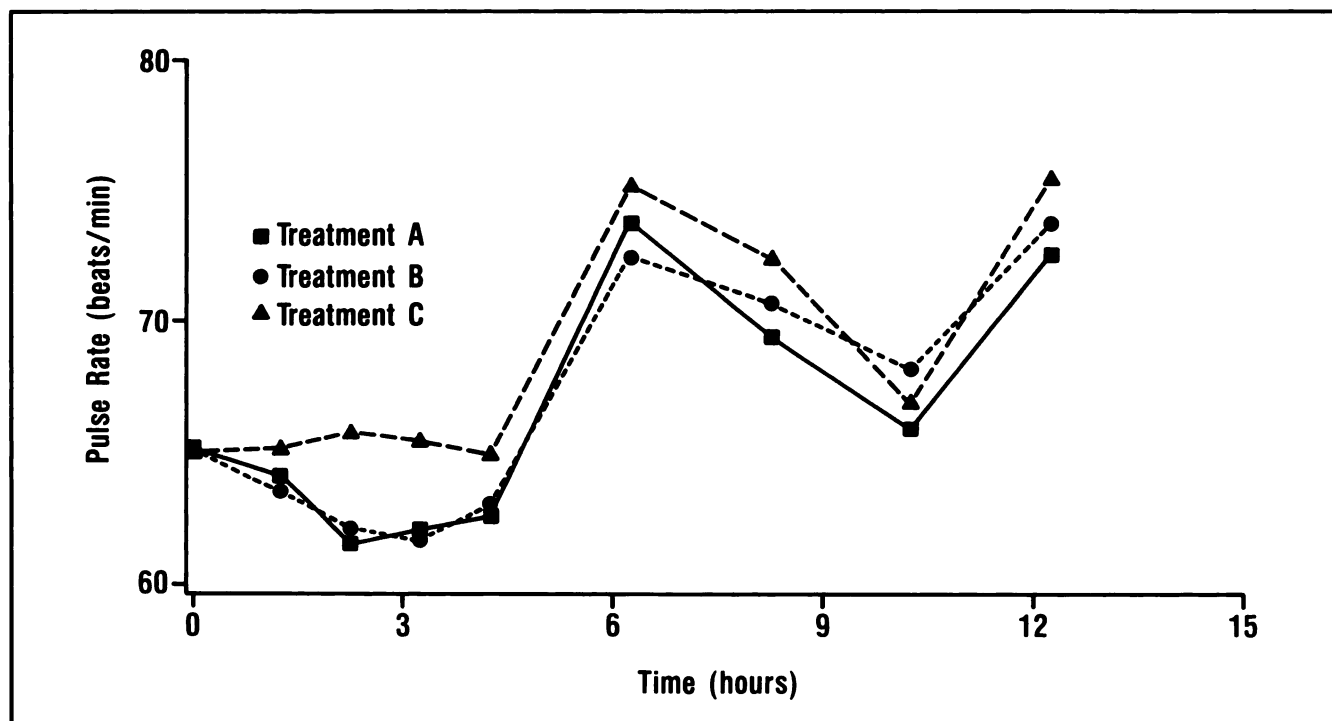


Figure 4. Mean supine pulse measurements after single oral doses of 2.5 mg (treatment A), 5.0 mg (treatment B), and 10.0 mg (treatment C) minoxidil in 30 normal volunteers.

sampling time, no significant treatment effects were observed. Significant time effects within treatments were observed, but the differences were generally less than 10%. These observations agree with previous results^{1,9} indicating that minoxidil has little effect on blood pressure in normotensive subjects. Animal experiments, however, have shown that high doses of minoxidil, up to 20 mg/kg, exert hypotensive effects in normotensive rats, dogs, and rhesus monkeys.^{10,11} It is possible that blood pressure effects in normotensive subjects might also be observed with administration of higher doses of minoxidil. Treatment effects on pulse rate were significant at 2.25 and 3.25 hours ($P = 0.0005$ and $P = .0147$, respectively), but the differences between treatments were less than 10%. Significant time effects were observed within each treatment for pulse rate, with the pulse rate significantly elevated compared with baseline at 6.25, 8.25, and 12.25 hours for each treatment ($P < .05$ in all cases). There are two explanations for this observation: (1) a natural circadian effect on blood pressure or (2) a transient decrease in blood pressure caused by minoxidil which is compensated for by an increase in heart rate. Based on the available data, the exact reason for the observed elevation in supine pulse cannot be determined.

In conclusion, the results of this experiment support dose-independent pharmacokinetics for min-

oxidil over the range of doses studied. These doses of minoxidil have little effect on blood pressure in normotensive volunteers.

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