

Pharmacokinetic studies of minoxidil

The disposition of minoxidil, a new direct arterial vasodilator and potent antihypertensive, was studied in 7 hypertensive patients. Following oral administration of ^{14}C -labeled minoxidil, there was a rapid removal of the drug with a plasma half-life of 4.2 hours. The radioactive label was recovered quantitatively in the urine, predominantly in the form of 3 metabolites. The major metabolite in the first 12 hours after giving the drug was a glucuronide conjugate. The other 2 metabolites have not been identified but appeared to be excreted predominantly after the first 12 hours, when minoxidil had been essentially cleared from the circulating blood. These observations suggest a dissociation between the drug in circulating blood and its pharmacologic activity. Since the prolonged hypotensive response to minoxidil cannot be attributed to slow removal, we suggest that it may be exerting a persistent effect on receptor sites.

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Minoxidil (6-amino-1,2-dihydro-1-hydroxy-2-imino-4-piperidinopyrimidine) has been shown to exert an antihypertensive effect,^{3, 6} which appears to be due to direct smooth muscle vasodilation† (Fig. 1). It has been compared to hydralazine and has been shown to be substantially more effective.⁶ Although observations in experimental animals suggested rapid clearance of the drug,⁹ no information regarding its fate was available in man. Therefore, we have undertaken studies with the use of radiolabeled

minoxidil in a small group of hypertensive patients.

Methods

Seven hypertensive patients were studied during hospitalization on a metabolic research ward. They were selected because of poor therapeutic responses to conventional antihypertensive therapy. Cardiovascular complications, including a variable degree of renal impairment, were present in all patients (Table I). In the initial period of hospitalization, during which the patients were treated with propranolol and hydrochlorothiazide, control supine blood pressures averaged 193/126 mm. Hg; this was reduced to 136/90 mm. Hg after 6 to 10 days during administration of minoxidil (2 to 30 mg. per day). Radioactive minoxidil, which was labeled with ^{14}C located in the 3 posi-

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Table I. Pharmacokinetic analysis of the disposition of minoxidil

Patient	Blood pressure (mm. Hg)		GFR (ml./min.)		Pharmacokinetic data			
	Control	Mnx	Control	Mnx	K_e (hr. ⁻¹)	V_D (L.)	Cl_{tot} (ml./min.)	Cl_R (ml./min.)
Sch.	(198/126)*	(198/126)	(26)	(26)	(.142)	(183)	(435)	(41)
	198/126	148/88	26	21	.200	101	357	42.3
Bal.	205/121	148/88	12	8	.095	329	519	10.0
Kla.	221/149	149/99	14	11	.024	99	265	13.8
You.	(155/115)	(155/115)	(57)	(57)	(.107)	(826)	(1480)	(64.9)
	155/115	127/90	57	59	.189	321	1020	76.1
Pul.	204/136	129/89	30	31	.136	214	484	38.2
Sel.	175/116	139/90	55	80	.306	208	1063	81.9
Hur.	193/122	142/91	130	142	.195	127	412	266
Mean	193/126	136/90	45	50	.163	200	588	73.9

*The figures in parentheses represent data obtained before blood pressure lowering with minoxidil (Mnx). K_e = fractional rate of decrease of plasma concentration of minoxidil. V_D = volume of distribution. Cl_{tot} = total clearance. Cl_R = renal clearance. GFR = glomerular filtration rate. The mean determinations are based on values during the treated periods only.

tion of the pyrimidine ring with a specific activity of 8.33 μ c per milligram, was used for these studies. It was administered orally to all patients after an overnight fast in a dose of 6.0 mg. (except patient B. A. L. who received only 3.0 mg.) after dissolution in 100 ml. of distilled water. Two patients received labeled drug prior to any treatment with minoxidil and then received a second dose as well after the maximum therapeutic response to minoxidil had been achieved. The other 5 patients received radioactive drug only under the latter circumstance. Appropriate blood and urine specimens were obtained together with 72 hour stool collections. Renal clearance of inulin was measured in all patients before and during the blood pressure reduction on minoxidil.⁷

Plasma, urine, and stool specimens were counted directly for total radioactivity. We also measured specific minoxidil radioactivity by an extraction technique in plasma and urine specimens. The efficiency of separation of minoxidil from its metabolites in urine by this method was demonstrated to be greater than 99 per cent by paper chromatography of the methylene dichloride extracts. All samples were analyzed in a liquid scintillation spectrometer with a dioxane counting solution²; internal standards were added for appropriate quench

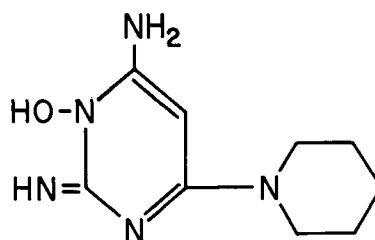


Fig. 1. Structure of minoxidil.

correction. Plasma minoxidil concentration curves were treated by least-squares analysis to obtain the fractional disappearance constant (K_e) during the terminal exponential phase. The slope of the terminal portion of the curve was extrapolated to zero time and the volume of distribution (V_D) calculated from the extrapolated initial concentration and the administered dose. The clearance from this volume of distribution was then calculated from V_D and K_e .⁴

Ascending chromatography of urine aliquots was carried out (Whatman No. 1; butanol:piperidine:water = 81:17:2) and analyzed for radioactivity in a paper strip radiochromatograph detector computing the relative amount of radioactivity as minoxidil and as each of its metabolites by planimeterization. Urines were chromatographed also after incubation in the presence of beta glucuronidase.

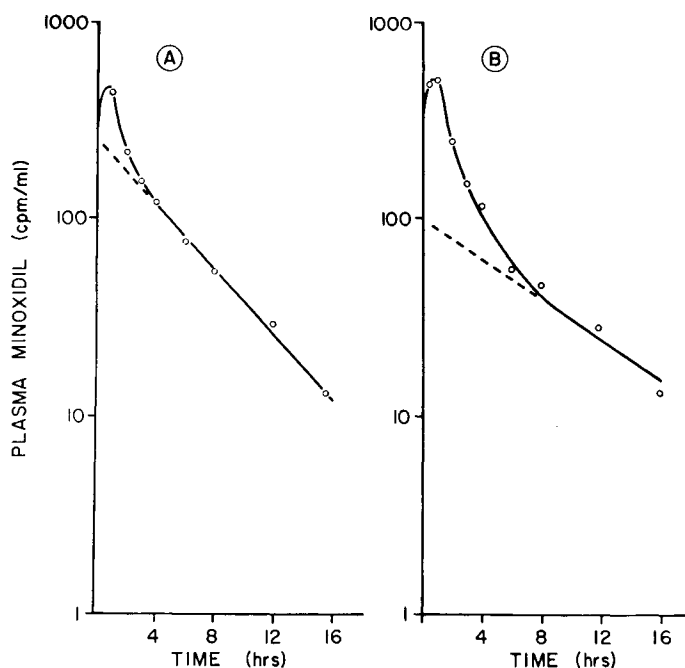


Fig. 2. Plasma minoxidil radioactivity in Patient You. The plasma concentration curve on the left (A) was obtained when the blood pressure had been lowered after 7 days' treatment with minoxidil. The curve on the right (B) was obtained before administration of minoxidil when the blood pressure was elevated. The same amount of ^{14}C -labeled drug was given in each case.

Results

Minoxidil appeared in the plasma samples within 0.5 hours after oral administration, and maximal concentrations occurred at one hour (Fig. 2, A). After an initial distribution phase, there was a slower decline which appeared to be exponential during the ensuing 4 to 16 hours, after which no radioactivity as minoxidil could be detected in plasma. Analysis (Table I) of plasma disappearance indicated that minoxidil was removed from plasma with a rate constant which averaged 0.163 hours^{-1} , consistent with a mean half-life of 4.2 hours. The volume of minoxidil distribution calculated from these data exceeded that of body water, averaging 200 L.; clearance was 588 ml. per minute.

In 2 patients, in whom studies were performed before therapeutic doses of minoxidil had been administered as well as after blood pressure lowering by 7 to 10 days' use of the drug, the disappearance rate constant was faster in the second phase

(0.200 and 0.189) than in the first (0.142 and 0.107 hours^{-1}) (Fig. 2, A and B). However, the volumes of distribution were both smaller during the second phase and the resulting total clearances were similar (Table I). In contrast to the rapid total clearance, renal clearance of minoxidil was approximately 10 per cent of this value, averaging 73.9 ml. per minute. Thus, renal disposition did not contribute substantially to the total body clearance of the drug.

Renal clearance of minoxidil was correlated with the glomerular filtration rate in these patients and in 6 of 7 patients was approximately equal to the calculated filtration rate. Renal function varied in patients between essentially normal values and values consistent with uremia (142 to 8 ml. per minute). Minoxidil was found not to be bound to plasma proteins by *in vitro* equilibrium dialysis studies and therefore was freely available for excretion.

Recovery of administered minoxidil was complete; 97 ± 2 per cent appearing in

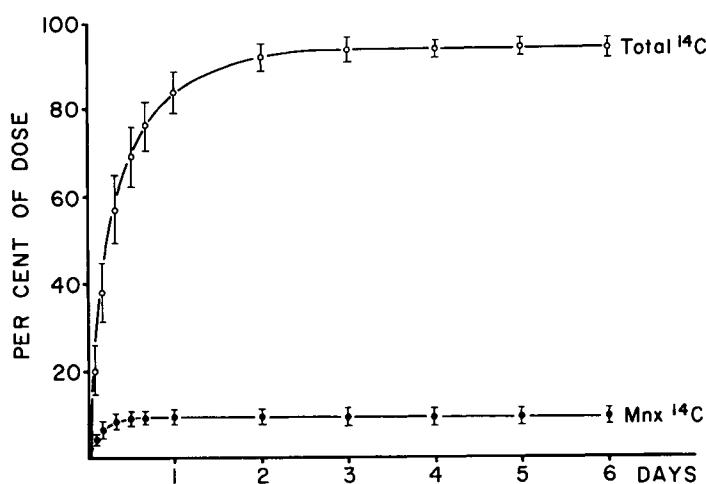


Fig. 3. The average cumulative excretion of ^{14}C in the urine after ^{14}C -minoxidil in 7 patients.

urine and 3 ± 1 per cent in feces (Fig. 3). Within 24 hours, over 80 per cent of the radioactive dose was recovered from urine. The remainder accumulated in the urine over the subsequent 4 days. Only a small fraction of the total dose was recovered in the urine as unmetabolized minoxidil (9.8 ± 7 per cent), and this had accumulated within the first 24 hours.

Three major metabolites of minoxidil were defined by radiochromatography of the urine. The radiochromatograms of early urine samples demonstrated the presence of minoxidil with an R_f of 0.7, together with metabolites whose radioactive peaks appeared at R_f values of 0.1, 0.2, and 0.3. These four peaks could also be defined in an acidic chromatographic system. One metabolite was identified as a glucuronide conjugate by enzymatic hydrolysis of the urine and subsequent radiochromatography of the hydrolysate. The other two metabolites (M-I and M-II) were not positively identified, although they appeared to be acidic in nature since their migration was enhanced in the acidic solvent system.

The relative contribution of minoxidil and its metabolites to the total urinary excretion of radioactivity suggested that the unchanged drug and its conjugate were excreted as the dominant substances in the first 8 hours and M-I and M-II were dominant thereafter (Fig. 4). Rates of elimi-

nation expressed as per cent per hour were also calculated for minoxidil, the glucuronide, and the two other metabolites (Fig. 5). During the first 24 hours, when approximately 80 per cent of the total dose had been recovered in urine, minoxidil accounted for $9.7 \pm .7$ per cent of the dose, the glucuronide for 38.5 ± 4.3 per cent, M-I for 17.1 ± 1.5 per cent, and M-II for 14.6 ± 1.7 per cent. Expressed as the fraction of total dose, the glucuronide was the dominant metabolite during the first 12 hours, whereas M-I and M-II were in greater concentration thereafter.

Discussion

The plasma half-life of 4.2 hours of minoxidil appears to be as rapid in our hypertensive patients as it was in experimental animals.⁸ The large volume of distribution of the drug suggested that concentration must occur at certain extravascular sites in body.

There was a difference in the disposition of minoxidil before and during treatment when a hypotensive effect had been achieved. Although the plasma disappearance was faster in the second phase, the volume of distribution was smaller and the calculated clearance from this volume was essentially unchanged. It would appear that prior administration of minoxidil or the achievement of a hypotensive response

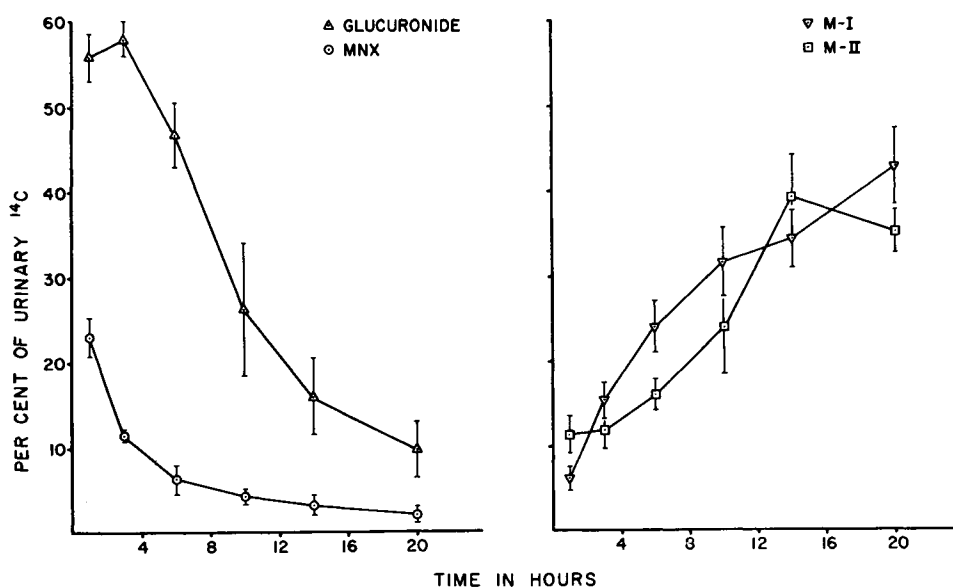


Fig. 4. Excretion of minoxidil (*Mnx*), its glucuronide conjugate, M-I, and M-II in urine during 24 hours after minoxidil. These fractions are each expressed as a fraction of the total ¹⁴C activity in the urine sample at the specified time.

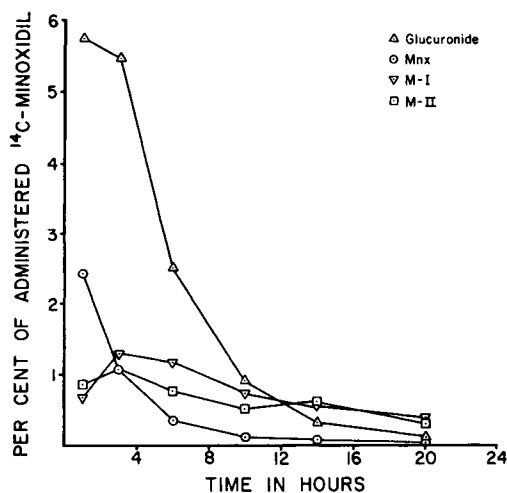


Fig. 5. Excretion of minoxidil (*Mnx*), its glucuronide conjugate, M-I, and M-II in urine expressed as the fraction of the total ¹⁴C-minoxidil administered to the patient (per cent per hour).

alters the distribution of the drug without significantly increasing the rate at which it is cleared from the body.

Our data do not explain whether the decreased volume of distribution is the consequence of an altered perfusion pattern within the cardiovascular system as a result of the hypotensive effect of minoxidil

or of some other factor. In all patients, the total clearance of minoxidil greatly exceeded the renal clearance, suggesting a predominate role of metabolism in its disposition. However, we have no evidence to indicate that minoxidil induces the enzymatic system responsible for its metabolism in a period as short as 7 to 10 days. The renal clearance of minoxidil approximated or slightly exceeded that of inulin, indicating that drug which was not bound to plasma protein was excreted by the kidney, principally by filtration without a significant renal tubular contribution.

The removal of minoxidil from plasma was rapid relative to the duration of hypotensive activity. Recently, we⁶ indicated that this action may exceed 24 hours in hypertensive patients. In view of the short plasma half-life this may seem unusual. However, if the drug accumulated at receptor sites, it would explain the prolonged hypotensive effect. Studies in rats using radioactive minoxidil have suggested that there may be retention of the drug in arterial vascular tissue.⁸ If the change induced in vascular tissue resulting from the drug receptor interaction was more persistent

than the minoxidil at the receptor site, it would account for the disparity between plasma half-life and duration of hypotensive effect.

The metabolic pattern of disposition of minoxidil was found to vary with time after administration. A glucuronide conjugate of minoxidil was observed as the predominant metabolite in the urine in the earlier collection periods; its presence in urine appeared to correlate with the presence of unmetabolized minoxidil in the urine or plasma during the first 12 hours. Thereafter, the other metabolites of minoxidil were predominant. Their late appearance leads us to speculate whether these metabolites may arise from minoxidil at receptor sites where the drug is retained, whereas the metabolism of minoxidil circulating in the blood results in the glucuronide.

The glucuronide conjugate is the only metabolite so far identified. Oxidation of the piperidine ring might be another pathway of metabolism, since this is a major pathway in the dog, with hydroxylation at the 3 and 4 positions.* However, the hypertensive patients whom we studied did not appear to produce either of these compounds. The human metabolism of another vasoactive drug, dipyridamole, with a piperidinopyrimidine structure has been demonstrated to involve solely glucuronide conjugation with no identifiable hydroxylated metabolites.¹ Indeed, review of other drugs with a piperidine structure does not support its oxidation.^{5, 9} The chemical structure and the pharmacologic activity of the metabolites, M-I and M-II, would provide important information.

The clinical implication of our pharmacokinetic and metabolic data is that the disposition of minoxidil depends primarily on metabolism and only secondarily on renal excretion of unchanged drug. Thus, plasma disappearance or total body clearance of

the drug is not a function of the level of glomerular filtration rate and neither drug levels nor pharmacologic activity would be expected to accumulate excessively in patients with azotemia. This is important, since minoxidil has been used in patients refractory to conventional antihypertensive drug therapy in whom renal function may be compromised. These studies also indicate that the pharmacologic activity of minoxidil may not relate directly to its presence in plasma. We would suggest that the prolonged hypotensive response to the drug may reflect the fate of the drug at tissue receptor sites.

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