small degree of transformation of isophenindamine to phenindamine occurred after standing in the mobile phase for ~1 day.

To take advantage of the properties of silver nitrate, it was necessary to ensure that all materials in contact with the mobile phase were made of 316 stainless steel or other noncorrodible material. The detector balance had to be electronically offset to monitor the effluent at 254 nm because of the relatively high absorbance of the mobile phase. By observing these relatively simple directions and precautions, the system was safe and reliable, producing no column deterioration over ~3 months of use.

REFERENCES

(1) G. Lehmann, *J. Pharmacol. Ther.*, **92**, 249 (1948).

(2) J. Plati and W. Wenner, *J. Org. Chem.*, **20**, 1412 (1955).

ACKNOWLEDGMENTS

The authors thank Dr. J. Plati for the isophenindamine nitrate reference standard, Mr. N. Beratti for spectroscopic data, and Ms. G. Capitano for technical assistance.

Effect of Exercise on Renal Clearance of Atenolol

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Received April 30, 1979, from the **Pharmacokinetics Laboratory, Schools of Pharmacy and Medicine, University of Missouri-Kansas City, Kansas City, MO 64108,* and the ‡*Clinical Research Department, ICI Americas, Inc., Wilmington, DE 19897.* Accepted for publication October 1, 1979.

Abstract □ The effect of exercise on plasma atenolol was evaluated using a two-phase complete crossover study in 12 healthy volunteers. In one phase, the volunteers were subjected to physical exertion on a treadmill; in the other phase, they remained in a sitting or standing position. Following a single 100-mg atenolol dose, frequent blood samples and a complete urine collection were obtained over 24 hr. Plasma and urine atenolol levels were assayed by high-pressure liquid chromatography. Plasma atenolol was significantly ($p < 0.05$) higher during the exercise phase of the study, and this result was associated with approximately an 8% decrease in the renal clearance of the drug, probably due to decreased renal blood flow during exercise.

Keyphrases □ Atenolol—effect of exercise on renal clearance □ Pharmacokinetics—atenolol, effect of exercise on renal clearance □ Renal clearance—atenolol, effect of exercise □ β-Adrenergic blocking agents—atenolol, effect of exercise on renal clearance

Recently published reports described the pharmacokinetics (1) and pharmacodynamics (2) of atenolol, 2-[*p*-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide, a new cardioselective (3–5) β-adrenergic blocking agent. More than 90% of the bioavailable dose of atenolol is excreted unchanged in the urine within 48 hr of dosing (6). To study the relationship of pharmacokinetics to pharmacodynamics, a two-phase complete crossover design was employed, with the phases differing only in the amount of exercise required of the subjects. A difference in the kinetics of atenolol between the two phases (exercise *versus* nonexercise) is reported.

EXPERIMENTAL

The 12 healthy male volunteers were 20–28 years old and weighed 63.6–85.5 kg (140–188 lb). Each subject was judged healthy based on the absence of any abnormality in his history, physical examination, ECG, hemogram, blood chemistries, and urinalysis.

The study design was a two-phase complete crossover, with the treatments being identical except for the exercise performed by each subject. Following a 10-hr fast, each subject received 100 mg of atenolol as an oral solution in 240 ml of water. The fast continued until after the 4th-hr blood sample was drawn; a standard meal was served at the 5th hr. Following the meal, water was allowed *ad libitum*. Blood samples were collected at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, and 24.0 hr. Urine was collected prior to dosing, from 0.0 to 12.0 hr, and from 12.0 to 24.0 hr.

Plasma and urine atenolol levels were determined by a high-pressure liquid chromatographic (HPLC) method described previously (7) and modified as follows. The mobile phase consisted of a 4.00 m*M* solution of 1-heptanesulfonic acid sodium salt, 1.0% acetic acid, and 60.0% methanol in distilled water. The mobile phase was pumped at 1.5 ml/min and 20° through a stainless steel column packed with a high efficiency bonded-phase packing[1]. Aliquots of 150 μl of the reextraction solution were injected directly on-column through the injector. The atenolol peak was well separated from the procainamide internal standard peak and from artifacts. The retention times of atenolol and procainamide were 5.8 and 12.3 min, respectively.

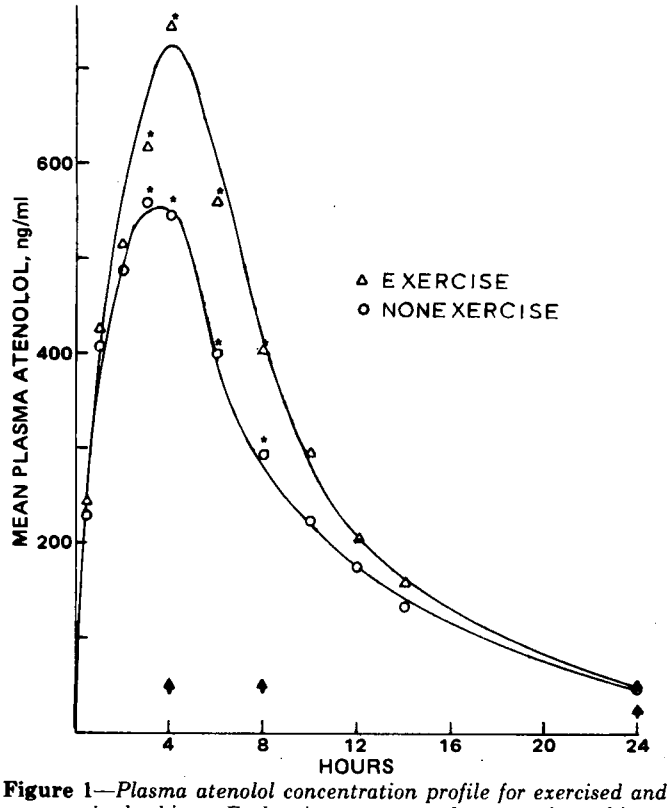

Figure 1—*Plasma atenolol concentration profile for exercised and nonexercised subjects. Each point represents the mean of 12 subjects. Starred (*) points represent significant differences ($p < 0.05$) between exercised and nonexercised subjects. Arrows indicate times of exercise.*

[1] Spherisorb 5-μm ODS, Laboratory Data Control, Riviera Beach, Fla.

0022-3549/80/0300-0344$01.00/0

Table I—Bruce Protocol

Stage	Speed, mph	Grade, %	Duration, min	Total Time Elapsed, min
1	1.7	10	3	3
2	2.5	12	3	6
3	3.4	14	3	9
4	4.2	16	3	12

Table II—Plasma Atenolol Concentrations for Nonexercise Treatment *versus* Exercise Treatment

Hours	Nonexercise, ng/ml Mean	Nonexercise, ng/ml *SD*	Exercise, ng/ml Mean	Exercise, ng/ml *SD*	Statistics[a]
0.0	0.0	0.0	0.0	0.0	
0.5	229	94.1	244	200	NS
1.0	408	105	428	171	NS
2.0	488	161	515	205	NS
3.0	560	228	618	204	NS
4.0	547	188	746	222	S
6.0	400	168	561	157	S
8.0	296	89.5	403	105	S
10.0	226	72.7	297	79.0	S
12.0	177	56.3	207	53.4	NS
14.0	135	43.9	161	41.9	NS
24.0	49.1	13.3	51.6	21.2	NS
$AUC^{0\rightarrow 24}$[b], μg hr/ml	5.53	1.79	6.85	1.74	S
X_u^{24}[c], mg	49.4	15.7	56.1	12.3	NS
X_u^{12}[d], mg	39.4	13.4	42.0	13.3	NS
Cl_R[e], ml/min	150	18.6	139	14.3	S

[a] S denotes significant difference at 0.05 significance level by analysis of variance; NS denotes no significant difference. [b] Area under the plasma concentration–time profile from 0 to 24 hr. [c] Amount of drug collected in the urine in 24 hr. [d] Amount of drug collected in the urine in 12 hr. [e] Renal clearance = $X_u^{0\rightarrow 24}/AUC^{0\rightarrow 24}$ hr.

During the nonexercise phase, the subjects remained in a sitting or standing position while confined to a small room. Subjects were confined for the entire 24-hr period of each phase of the study. The exercise phase consisted of the four stages of the Bruce protocol (8) (Table I) prior to dosing and at 4, 8, and 24 hr following each dose.

The mean heart rate for the 12 subjects prior to atenolol administration was 174 beats/min at Stage 4 of exercise. Volunteers in the exercise group were subjected to further physical exertion by the need to walk from their location up one flight of stairs to the treadmill, a distance of 114.3 m (375 ft). The subjects then rested until a resting pulse was attained, and treadmill exercise was then performed.

RESULTS AND DISCUSSION

Table II and Fig. 1 present the mean plasma and urine atenolol with the individual and mean areas under the plasma–time profiles and the calculated renal clearances for each treatment with the corresponding statistics. Analysis of variance showed differences at the 4-, 6-, 8-, and 10-hr times in plasma atenolol ($p < 0.05$) such that plasma atenolol was higher during exercise. The $AUC^{0\rightarrow 24}$ and Cl_R also showed significant ($p < 0.05$) differences. All other times and $X_u^{0\rightarrow 24}$ did not show significant differences. Since the total amount of atenolol excreted in 24 hr did not differ between exercised and nonexercised subjects and the area under the curve ($AUC^{0\rightarrow 24}$) was significantly increased during exercise ($p < 0.05$), the computed renal clearance decreased during exercise.

With the commencement of exercise at 4 hr, a change in plasma atenolol was attained. This result is consistent with the fact that the change in $AUC^{0\rightarrow 24}$ and Cl_R with exercise is due to the exercising condition and not some other factor. Urinary recovery of atenolol verified previous recoveries (1), consistent with published results showing orally administered atenolol to be 0.58 ± 0.16 bioavailable.

The decrease in renal clearance of atenolol of ~8% can possibly be explained by changes in renal plasma flow. Decreases of up to 35% normal renal plasma flow were noted in a similar treadmill test as the Bruce treadmill protocol (9–11). During exercise, neurogenic and hormonal influences shunt blood away from the kidney to other areas (*i.e.*, heart, brain, and skeletal muscle). Since atenolol is essentially totally eliminated *via* the kidney, a decrease in renal clearance, Cl_R, would be expected during exercise.

A review (12) of the interrelationships among renal hemodynamics, drug kinetics, and drug action described the potential effects of reduced renal blood flow on the clearance and distribution of drugs but did not directly address the potential effects of exercise. Little is known of the effects of exercise on drug kinetics. An 8% increase in plasma atenolol during exercise would probably not be therapeutically significant, but it does demonstrate the importance of controlling exercise levels during pharmacokinetic and bioavailability studies.

REFERENCES

(1) W. D. Mason, N. Winer, G. Kochak, and I. Cohen, *Clin. Pharmacol. Ther.*, **25**, 408 (1979).

(2) N. Winer, W. D. Mason, C. H. Carter, T. L. Willoughby, G. Kochak, I. Cohen, and R. Bell, *ibid.*, **26**, 315 (1979).

(3) A. M. Barret, J. Carter, J. D. Fitzgerald, R. Hull, and D. DeCount, *Br. J. Clin. Pharmacol.*, **2**, 527 (1975).

(4) J. D. Harry, M. F. Knapp, and R. J. Linder, *Br. J. Pharmacol.*, **48**, 340 (1973).

(5) R. Hainsworth, F. Karim, and J. B. Stocker, *ibid.*, **48**, 342 (1973).

(6) J. C. Brown, S. G. Carruthers, G. D. Johnston, J. G. Kelly, J. McAnish, D. G. McDevitt, and R. G. Shanks, *ibid.*, **20**, 524 (1976).

(7) O. H. Weddle, E. N. Amick, and W. D. Mason, *J. Pharm. Sci.*, **67**, 1033 (1978).

(8) R. A. Bruce, J. R. Blackmon, J. W. Jones, and G. Strait, *Pediatrics, Suppl.*, **32**, 742 (1963).

(9) C. B. Chapman, A. Henschel, J. Minckler, A. Forsgren, and A. Keys, *J. Clin. Invest.*, **27**, 639 (1948).

(10) G. Grimby, *J. Appl. Physiol.*, **20**, 1294 (1965).

(11) C. B. Chapman, A. Henschel, and A. Forsgren, *Proc. Soc. Exp. Biol. Med.*, **69**, 170 (1948).

(12) K. L. Duchin and R. W. Schrier, *Clin. Pharmacokinet.*, **3**, 58 (1978).