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Report

Comparison of Single-Dose and Steady-State Nadolol Plasma Concentrations

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The pharmacokinetics of nadolol have been previously reported to be linear between single and steady-state dosing. Data from a study in our laboratory suggested greater than expected β-blockade with nadolol at steady state. Because the early potency studies were single-dose studies, we hypothesized there was a nonlinearity in nadolol pharmacokinetics which produced higher than expected plasma concentrations at steady state. Six normal volunteers from the previous study (steady state) volunteered to participate in the single-dose study. Plasma concentrations were determined for 24 hr following a single dose of nadolol, 80 mg. A simple, inexpensive, and accurate method for determination of nadolol in plasma or serum by HPLC with fluorometric detection is described. The AUC_{0-tau} at steady state was greater than the $AUC_{0-\infty}$ following a single dose in five of the six subjects. The mean ratio of AUC_{ss}/AUC_{sd} was 2.54. This value would be unity in the presence of linear pharmacokinetics. We conclude that the principle of superposition is not applicable for nadolol.

KEY WORDS: nadolol; pharmacokinetics; high-performance liquid chromatography (HPLC); nonlinearity; β-blocker.

INTRODUCTION

Nadolol (Corgard), 2,3-*cis*-5-[3-(1,1-dimethylethyl)-amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol, is a nonselective β-blocker with no intrinsic sympathomimetic activity and no membrane stabilizing activity (1). Although it is a β-blocker commonly used in the treatment of hypertension and angina pectoris, very few studies have been performed on the drug's pharmacokinetics in single or multiple doses. The studies which have been performed report a total-body clearance of about 200 ml/min, a volume of distribution of about 2 liters/kg, elimination half-lifes ranging from 12 to 24 hr, a bioavailability of approximately 30%, and 30% binding to plasma proteins (2–4). There is no evidence of metabolic elimination, although some of the drug may be excreted in bile (2).

In a recent study, we compared the effects of propranolol and nadolol on β-receptor regulation on lymphocytes and on the time course of hyperresponsiveness to adrenergic stimulation following abrupt β-blocker withdrawal in eight normal volunteers (data to be published). In all subjects we noted that the degree of β-blockade achieved following the daily administration of nadolol, 80 mg, was considerably greater than we saw with propranolol, 40 mg given every 6 hr. We had expected the β-blocking activity of the selected doses of these two drugs to be approximately equal, based on oral potency data (1,5). As the potency data were based on single-dose studies, we hypothesized that nadolol pharmacokinetics may not demonstrate superposition, resulting in greater than expected β-blockade at steady state. The objective of the present study was to test this hypothesis by performing a single-dose pharmacokinetic study of nadolol to compare with the previous steady-state study. We utilized a newly developed HPLC method to quantitate the serum concentrations of nadolol.

MATERIALS AND METHODS

Analytical

Extraction. Five hundred microliters of plasma or serum was pipetted into a 10-ml polypropylene test tube (Kew Scientific, Columbus, OH) along with 10 ng of the internal standard (D-617, a verapamil metabolite) and 500 μl of 5 *N* sodium hydroxide. Four milliliters of high-purity methyl-t-butyl ether (Burdick and Jackson, Muskegon, MI) was added and the mixture was shaken on a reciprocating shaker for 10 min, followed by a 10-min centrifugation. The aqueous phase was then frozen in a dry ice/acetone bath and discarded. The ether was decanted into an 8-ml polypropylene tube (Kew Scientific, Columbus, OH) and 50 μl of dilute aqueous sulfuric acid (pH 2.2) was added. This mixture was then shaken and centrifuged for 10 min each. The aqueous phase was again frozen in a dry ice/acetone bath and the ether layer was discarded. The aqueous phase was allowed

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to warm to room temperature and was then vortexed and injected on to the column. Extraction efficiency of nadolol was 91 and 94% at concentrations of 50 and 167 ng/ml, respectively. Extraction efficiency of the internal standard (D-617) was similar, at 93 and 95% at concentrations of 15 and 50 ng/ml, respectively.

Detection. A chromatogram of extracted blank plasma and nadolol extracted from plasma is shown in Fig. 1. Detection of nadolol and D-617 was accomplished with a fluorescence detector (Model 970, Kratos Analytical Instruments, Ramsey, NJ) at an excitation wavelength of 209 nm with an emission wavelength of 305 nm. The samples were injected onto a 20-μl loop and the drugs were resolved by a cyanopropyl column (4.6-mm ID × 25 cm, 5 μm particle size, Rainin Instrument Co., Inc., Woburn MA). The mobile phase consisted of 125 μl of 3 *M* sulfuric acid per liter of spectrograde methanol (Burdick and Jackson, Muskegon, MI). An isocratic pump (Beckman Model 114M, Beckman Instrument Co., Berkeley, CA) was used to pump the mobile phase at a constant rate of 1 ml/min. Detector output was simultaneously recorded on a linear chart recorder (Model B040, Kipp and Zonen, The Netherlands) and the recorder speed was 2 mm/min. The lower limit of sensitivity for the assay was 1 ng/ml.

Standards. Standard samples and quality controls were prepared by adding the appropriate amount of nadolol to outdated blood-bank plasma which was obtained from the American Red Cross (Columbus, OH). Within-day coefficients of variation were determined at concentrations of 15, 50, and 150 ng/ml and were 5.6, 3, and 2.7%, respectively. Ten samples of each concentration were evaluated to determine the variability. Day-to-day variability was determined by measuring 20 and 125 ng/ml quality control samples on 11 different days over a 3-week period. Day-to-day variability was 5.9 and 3.1% at concentrations of 20 and 125 ng/ml, respectively. Standard curves were constructed by plotting the peak height ratios of nadolol to internal standard versus known concentrations of drug standards. The unknown concentrations were determined by least-squares linear regression analysis (6). The accuracy of our new HPLC method is evident by comparing the area under the plasma concentration–time curve values that were obtained in this study with those from Dreyfuss and co-workers' study (3), which determined nadolol plasma concentrations with ^{14}C-nadolol. The within-day and day-to-day variability show that the method is also very precise.

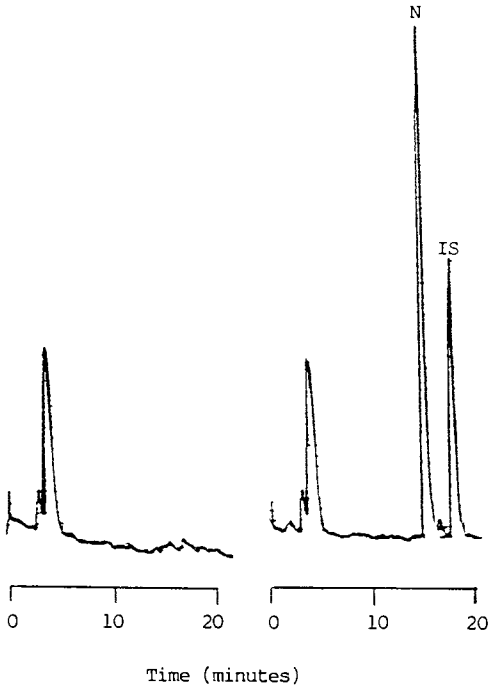

Fig. 1. Chromatograms of blank plasma and nadolol (N) and internal standard (IS) extracted from plasma. Time of injection is represented by the hatch mark at time zero.

Clinical Studies

Eight normal male volunteers aged 21 to 24 years (mean ± SD, 22.5 ± 1.3 years) participated in a study in which β-receptor density and response to propranolol and nadolol were determined. Pharmacokinetic parameters of the two drugs at steady state were also determined. The subjects signed informed written consent and the study was approved by the Human Subjects Review Committee at the Ohio State University; it was conducted in the Clinical Research Center at the Ohio State University Hospitals. The participants' healthy status was determined by physical examination, electrocardiogram, and routine laboratory studies. Their weights ranged from 73 to 84 kg (79.3 ± 4.1 kg). They were taking no other medications and they were all nonsmokers.

As part of the β-receptor density/response study, the subjects took nadolol, 80 mg, once daily for 7 days. Blood samples were collected 1, 2, 4, 8, 12, and 24 hr after the last dose of nadolol for determination of nadolol serum concentration. Blood samples were collected in tubes containing no anticoagulant or separator gel. Samples were allowed to clot and were then centrifuged; the serum was separated and stored at −20°C until evaluation. As described in the Introduction, the response to nadolol was much greater than expected, and a single-dose study was therefore undertaken. Six of the subjects participated in the single-dose study in which they took nadolol, 80 mg, and blood samples were drawn 1, 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hr after the dose. The other two subjects who participated in the study of nadolol at steady state were not available for participation in the single-dose study. In both the single-dose and the steady-state studies, doses were administered after an overnight fast and food was withheld for 3 hr after the dose.

Pharmacokinetics

The area under the plasma concentration time curve following a single dose ($AUC_{0-\infty}$) was calculated by the trapezoidal rule (7) with extrapolation to infinity using the terminal slope of the serum concentration–time curve, which was determined by linear regression analysis. The area under the nadolol serum concentration–time curve at steady state (AUC_{0-24}) was also calculated by the trapezoidal rule. Superposition was assessed by calculating the ratio of $AUC_{0-24}/AUC_{0-\infty}$. In the presence of linear pharmacokinetics and the superposition principle, the AUC values should be equal and the ratio should equal one.

A one-compartment first-order absorption model was

computer fitted by nonlinear least-squares regression analysis (8) to the mean nadolol serum concentration–time data following administration of the single dose. This was done so that a predicted steady-state plasma concentration–time profile could be determined by using the fitted parameters and the multiple-dosing function.

RESULTS

The arithmetic mean values for half-life were 10.4 ± 2.96 hr following a single dose and 11.6 ± 4.8 hr at steady state. These are similar to the harmonic mean values for half-life, which were 9.96 hr following a single dose and 10.1 hr at steady state.

Figure 2 shows the mean plasma concentration–time curves following a single dose and at steady state. The solid line represents the predicted steady-state plasma concentration–time profile based on single-dose data and incorporation of the multiple-dosing function. It is clear from Fig. 2 that the plasma concentrations achieved at steady state were much higher than predicted from the single-dose data. The values for $AUC_{0-\infty}$ (single dose), AUC_{0-24} (steady state), and the ratio of $AUC_{0-24}/AUC_{0-\infty}$ are shown in Table I. The differences in $AUC_{0-\infty}$ and AUC_{0-24}, compared by paired Student's t test, were of borderline significance ($P = 0.063$). However, when subject 5 is excluded from the analysis, the difference becomes highly significant ($P = 0.009$). In none of the subjects studied were the AUCs following single and multiple doses the same.

DISCUSSION

The principle of superposition enables one to determine the plasma concentration at a certain time following multiple dosing based on the plasma concentration at that same time following a single dose and by incorporating the multiple-dosing function (9). This principle assumes that the drug exhibits linear pharmacokinetics and that the $AUC_{0-\infty}$ following a single dose is equal to the AUC_{0-tau} following steady-state dosing, where tau is the dosing interval.

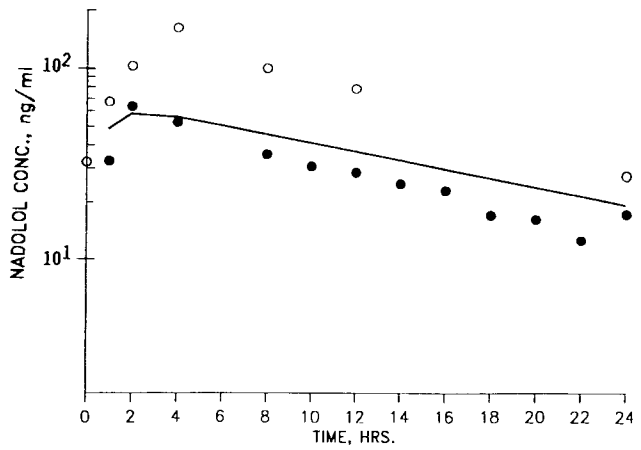

Fig. 2. Plasma concentration–time curves of nadolol following single dose and steady state. Filled symbols represent mean plasma concentrations following a single dose of nadolol. Open symbols represent mean plasma concentrations at steady state. The solid line represents the predicted steady-state plasma concentrations assuming the principle of superposition.

Table I. Area Under the Curve Values Following Single and Multiple Doses of Nadolol, 80 mg[a]

Subject No.	$AUC_{0-\infty}$ (ng*hr/ml)	AUC_{0-24} (ng*hr/ml)	AUC_{SS}/AUC_{SD}
1	569	2785	4.89
2	1050	2672	2.54
3	1147	2131	1.86
4	295	858	2.91
5	1718	973	0.57
6	895	2195	2.45
Mean	946	1936	2.54
SD	493	832	1.41

[a] $AUC_{0-\infty} = AUC_{SD}$, $AUC_{0-24} = AUC_{SS}$.

Dreyfuss *et al.* (3) compared the plasma concentration–time profiles from single oral 2- and 10-mg nadolol doses (normalized to 80 mg) and single and multiple oral doses of nadolol, 80 mg. They described the plots as nearly superimposable and therefore concluded that the data established the superposition principle and that nadolol exhibited linear pharmacokinetics between 2 and 80 mg. The authors did not describe the relationship between the AUCs for these different doses and regimens. However, calculation of the AUCs following single and multiple dosing is possible from the data presented in their paper, and these calculations suggest that data in two of the three subjects who received both single and multiple doses did not confirm superposition.

In our study, nadolol pharmacokinetics failed to obey the superposition principle; the mechanisms responsible for this are not clear. There are several possible explanations for this observation, although the results of our study do not suggest which mechanism is most likely. The AUC is determined by bioavailability and clearance, therefore alterations in one of these parameters at steady state would result in differences between $AUC_{0-\infty}$ and AUC_{0-24}.

One explanation revolves around the clearance of nadolol, which is almost entirely dependent on renal processes. It is possible that nadolol undergoes concentration-dependent renal secretion, resulting in lower renal clearance at steady state than following single-dose administration. The relative consistency between the half-life at single dose and that at steady state would suggest that if clearance is decreasing upon multiple dosing, the volume of distribution is also decreasing to a similar extent. Because the contribution of secretion to the renal clearance of nadolol appears to be minimal (3), it seems unlikely that changes in renal secretion would result in the dramatic differences in $AUC_{0-\infty}$ and AUC_{0-24} shown in this study.

A second possible explanation for our findings involves bioavailability, which for nadolol is about 30% (3). Since the hepatic clearance of nadolol is negligible, hepatic first-pass metabolism is low, thus the bioavailability of nadolol is low because of poor absorption. The delayed peak observed at steady state compared to single dose (observed: 4 versus 2 hr) suggests that the absorption rate of nadolol was decreased at steady state. Whether or not this delayed absorption is associated with drug treatment is unknown. It is pos-

sible that because of an increase in mean absorption time, a higher fraction of the administered dose was absorbed, which resulted in a disproportionately higher AUC_{0-24} than that predicted by the superposition principle. Finally, it is possible that a combination of reduced clearance and increased bioavailability is responsible for the increased AUC observed at steady state in this study.

Data from this study show that nadolol does not obey the superposition principle. This means that one may not accurately predict steady-state plasma concentrations or response based on single-dose data. Early pharmacologic studies with nadolol were primarily single-dose studies, and it has been suggested that the potency ratio of nadolol compared to propranolol is approximately 2:1 (1,5). As stated previously, the determination of nadolol steady-state pharmacokinetics was part of a larger study in which the response to propranolol, 160 mg per day, and nadolol, 80 mg per day, was evaluated. Based on the data suggesting a potency ratio of 2:1, we expected to see approximately equal degrees of β-blockade with these two regimens. However, we saw 22% greater β-blockade with nadolol than with propranolol (unpublished data). Additionally, all of the study participants complained of more side effects (especially fatigue) with nadolol than with propranolol, clinical evidence of a greater degree of β-blockade with nadolol. The original potency ratios were based on single-dose data, and because the superposition principle is not satisfied by nadolol, dosing to steady state produces much higher plasma concentrations and therefore much greater β-blockade than would be expected from single-dose data.

ACKNOWLEDGMENTS

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