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The Assessment of the Beta-Blocking Activity of Urapidil: A New Method

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Summary. Urapidil is an antihypertensive vasodilator agent whose pharmacological action in man has not yet been fully defined. We have assessed the beta blocking activity of urapidil 15 mg and 30 mg i.v. in a single blind study of 10 healthy male volunteers. Urapidil at plasma concentrations in the same range as those shown to have antihypertensive affect did not significantly attenuate the chronotropic effect of isoproterenol. Propranolol 5 mg iv, the positive control, significantly shifted the isoproterenol dose-response curve to the right. We describe a new method of analyzing incomplete dose response curves whereby a linear terminal segment can be reproducibly defined.

Key words: urapidil; volunteers isoproterenol dose response analysis

Urapidil, [6-(-3-(4-(0 methoxyphenyl) piperazinyl-propylaminol) -1, 3-dimethyluracil], is a relatively new antihypertensive agent which was first introduced into clinical practice in West Germany in 1981. A number of studies have documented its efficacy in essential hypertension of all grades and in hypertension during anesthesia [1-5].

The primary actions of urapidil in animals are antagonism of peripheral α_1-receptors [6] and a central sympathetic-inhibiting effect [7] which, unlike that of clonidine, does not appear to be mediated via central α_2-receptor agonism.

Urapidil's pharmacological action is not yet fully defined in man. Recent studies have shown that the drug reduces both ventricular afterload and preload, thereby improving left ventricular performance, in healthy subjects [8] and in patients with congestive cardiac failure secondary to coronary artery disease [9].

In vitro studies have suggested that urapidil may have beta blocking action. Urapidil binds to cardiac $beta_1$-receptors [10] and weak competitive $beta_1$-antagonism has been demonstrated in guinea pig ventricular membrane [10], canine heart [11] and rabbit atria [12]. These findings have important implications for the drug's use in cardiac failure, when negative inotropic and chronotropic effects would clearly be undesirable.

A preliminary human study found that urapidil had no effect on exercise heart rate achieved during limited exercise at oral doses up to 37.5 mg [8]. To date, however, no study has examined the effect of urapidil on responses to iv isoproterenol in man.

The present study was designed to assess the beta blocking activity of intravenously administered urapidil in healthy volunteers.

Materials, Methods and Subjects

Approval for the study was given by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Ten hospitalized normotensive male volunteers (ages 22-31 years) were studied. All were in good health as determined by history, physical examination, electrocardiogram and standard blood and urine indices and were within 15% of ideal body weight.

No prescription or proprietary medications known to affect heart rate or blood pressure were permitted within two weeks prior to the study. Studies were conducted in a quiet, darkened room.

Following a control (placebo) study, subjects received urapidil 15 mg, urapidil 30 mg and propranolol 5 mg, in a randomized, single-blind fashion.

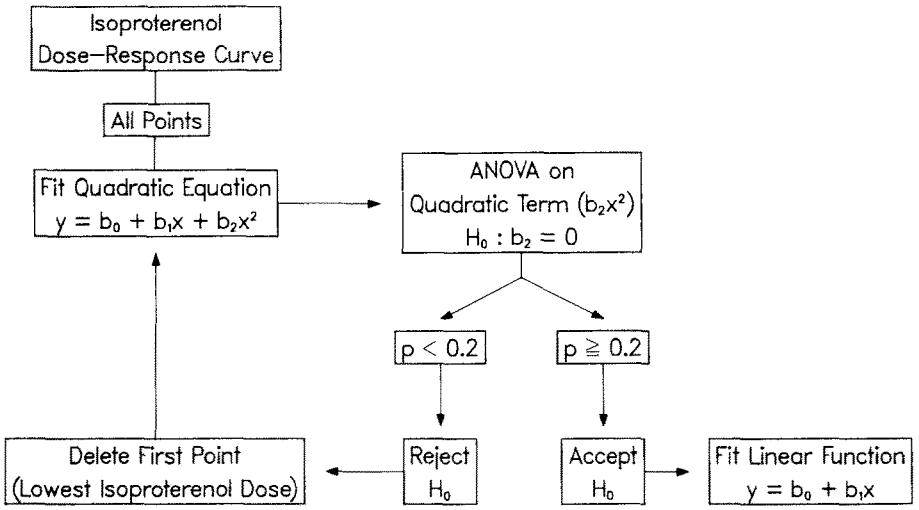

Fig. 1. Algorithm used in defining the terminal linear portion of an isoproterenol dose response curve (see text)

Active drug study phases were separated by at least 7 days. Subjects were fasted from 12 h before and until the end of each study. They remained in bed from 1 hour before drug administration until after the study when the absence of a significant orthostatic fall in blood pressure had been established.

Placebo, urapidil and propranolol were administered by square wave infusion through an indwelling venous cannula in the right arm. Placebo (15 ml of normal saline) and urapidil 15 mg (in 15 ml) were infused over 7.5 min, urapidil 30 mg (in 30 ml) was infused over 15 min and propranolol 5 mg (in 20 ml) over 20 min. The isoproterenol dose-response study was started 15 min after the end of each infusion. Isoproterenol intravenous boluses were administered over a maximum of 5 s into the iv giving set and flushed, via the giving set, with 5% dextrose-in-water infusion into a vein in the right forearm.

Heart rate response was measured from a continuous ECG paper trace. The three shortest consecutive R-R intervals in the 30 s prior to and in the 2 min after isoproterenol injection were compared to obtain the maximum heart rate response to each dose of isoproterenol. The maximum response usually occurred 60–90 s after the bolus dose. Increasing doses of isoproterenol were given at up to 5 min intervals until the heart rate response was at least 25 beats/min. On average, 10 doses of isoproterenol were given during placebo and urapidil phases and 14 doses during the propranolol phase. The mean durations of the isoproterenol periods after placebo, urapidil, and propranolol were 51, 52 and 92 min respectively.

On all but placebo days, blood samples were collected via an indwelling cannula in the left forearm for urapidil or propranolol concentrations. Samples were taken before, during and at the end of each drug administration and immediately before each isoproterenol dose.

Samples for urapidil assay were collected into 10 cc „venoject" evacuated glass tubes containing 143 units of sodium heparin. Samples for propranolol assay were taken using plastic syringes and transferred to collection tubes of the same type, from which the rubber stoppers had been removed and discarded [13]. Samples were centrifuged at 4 °C at 5000 g for 10 min within 30 min of collection and were immediately separated and stored. Urapidil samples were stored at −20 °C and propranolol samples at −70 °C until assay.

Plasma urapidil concentrations were determined by an HPLC method after liquid-liquid extractions from plasma. This method is specific for urapidil in the presence of its M1, M2 and M3 metabolites and isoproterenol. The limits of detection and quantitation are 1 ng/ml and 5 ng/ml respectively. The inter- and intra-assay coefficients of variation over the range of 5-1000 ng/ml are 6.6% and from 0.9–6.6% respectively. Plasma propranolol concentrations were measured using a radioimmunoassay technique described by Kawashima [14]. The antibody is highly specific for both *d*- and *l*-propranolol in the presence of propranolol metabolites. The limit of detection is 5 ng/ml and the intra and inter assay coefficients of variation are 6 and 11% respectively.

Isoproterenol dose response curves were constructed for each study day, and a linear terminal segment for each curve defined in the following manner (Fig. 1).

i) A quadratic function, $Y = B_0 + B_1x + B_2x^2$ (where Y = increase in heart rate over baseline, $x = \log_{10}$ dose of isoproterenol) was fitted by a non-weighted linear least squares method to all the data points.

ii) The significance of the quadratic term was evaluated by analysis of variance. If p was < 0.2 (F 1, n-3) the null hypothesis, $H_0 : B_2 = 0$ (that is, the quadratic

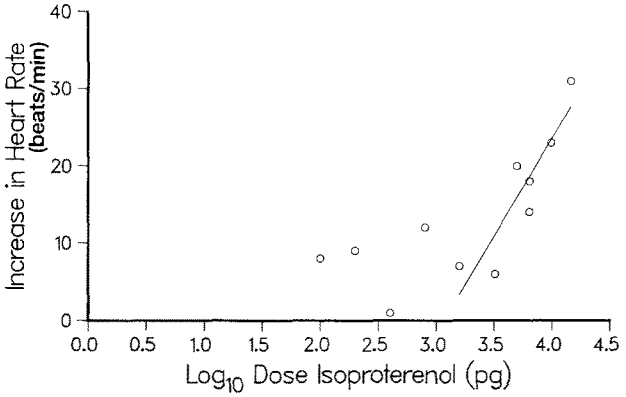

Fig. 2. Representative isoproterenol dose response curve (propranolol study) showing the best-fit terminal linear portion

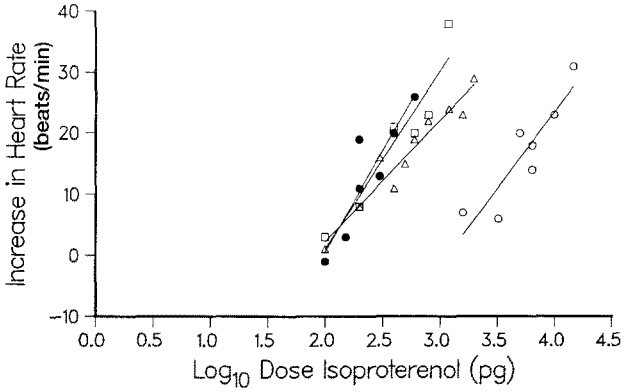

Fig. 3. Terminal linear portions of all four treatment days in one subject. Points which were deleted in defining the linear portions are not shown; △ Placebo; □ Urapidil 15 mg; ● Urapidil 30 mg; ○ Propranolol 5 mg

term, B_2x^2, did not contribute significantly to the regression) was rejected.

iii) If the null hypothesis was rejected the first point on the dose response curve was deleted, a quadratic function fitted to the remaining points and the F test repeated. Points were serially deleted until a p value of ≥ 0.2 was obtained. At this point the quadratic term was regarded as no longer contributing significantly to the regression.

iv) A linear function, $Y = B_0 + B_1x$, was then fitted to the remaining points. Steps i) to iv) were carried out using "Minitab" (Pennsylvania State University version 81.1) on a DECSYSTEM-20 computer. The average number of points used in the linear regressions on placebo, urapidil and propranolol days were 9, 8 and 8 respectively. To test for parallelism, blocked two way analysis of variance (Prophet/20 BBN Research Systems) was applied to the log-transformed slope values. Dose ratios for each urapidil dose and for propranolol were calculated from: ID_{20} (active drug)/ID_{20} (placebo), where ID_{20} is the dose of isoproterenol required to increase heart rate by 20 beats/min in the presence of active drug or placebo.

In order to assess whether the fitting procedure and choice of ID_{20} had biased the dose ratios, two additional methods of calculating dose ratio were used.

i) An 'empirical' ID_{20} for each subject was defined, by simply taking the smallest dose of isoproterenol that gave a heart rate increase of at least 20 beats/min.

ii) An ID_0, the "threshold" dose of isoproterenol was calculated by substituting for $y = 0$ in each linear regression. Again, dose ratio was calculated from ID_{20} (or ID_0) active drug/ID_{20} (or ID_0) placebo.

In order to assess whether falling plasma propranolol concentrations had contributed significantly to the slope of the response curves, multiple linear regression analysis was carried out with $\log_{10}$ propranolol concentration and $\log_{10}$ isoproterenol dose as determinants of heart rate response. If falling propranolol concentrations over the dose response period were reflected in significantly waning beta blockade we would expect a leftwards tendency, towards the placebo curve, of the upper end of the propranolol dose response; overall this would tend to steepen the propranolol curve. Multiple linear regression was not applied to the urapidil concentration data as the urapidil curves did not differ significantly from placebo. However, on both urapidil and propranolol study days a number of subjects were given the same low dose of isoproterenol at the start and at the end of the dose-response periods and the heart rate responses to these (equivalent) doses compared.

To assess the effect of each drug and placebo on resting heart rate, paired comparisons were made between heart rates measured immediately before and 15 min after each infusion. All paired comparisons were made using Student's *t*-test. One-tailed tests of significance were applied in the comparisons of dose ratios with unity. Two-tailed tests were used in all other comparisons. The 5% level was taken to indicate significance throughout.

Results

No changes were seen in resting heart rate after placebo and urapidil 15 mg infusions. Urapidil 30 mg and propranolol infusions were followed by small but statistically significant average increases (3 beats/min, $2p < 0.05$) and decreases (4 beats/min, $2p < 0.01$) in baseline heart rate respectively.

Figure 2 shows a dose response curve in a representative subject and indicates the best fit line for the terminal segment.

Table 1. Individual dose ratios of log dose isoproterenol vs increase in heart rate for drug intervention, compared to placebo. One-tailed t-tests of $\log_{10}$ transformed dose ratios compared with a mean of zero

Dose ratios			
Subject	Urapidil 15 mg	Urapidil 30 mg	Propranolol
1	1.4	2.2	11.2
2	13.2	6.4	85.9
3	1.0	0.6	1.2
4	0.5	2.8	11.0
5	1.5	1.1	11.7
6	1.5	11.1	47.7
7	1.6	2.1	12.0
8	0.3	1.4	40.1
9	0.6	0.5	9.4
10	2.0	0.7	17.4
Geometric mean	1.27	1.77	15.0
t	0.73	1.78	7.38
p	0.24	0.06	<0.0001

Figure 3 shows the terminal linear segments obtained in the same subject on each of the four study days.

There were no significant differences between placebo, urapidil and propranolol slopes for all subjects. ($F\ 3, 27 = 1.13$, $p = 0.337$)

The individual dose ratios and their geometric means are shown in Table 1.

Urapidil 15 and 30 mg dose ratios were not significantly different from 1, whereas propranolol dose ratios were significantly higher. Dose ratios calculated using the empirical ID_{20} method and ID_O method were not significantly different from those in Table 1. These methods gave rise, respectively, to dose ratios of 1.19 and 1.20 for urapidil 15 mg, 1.58 and 0.93 for urapidil 30 mg, 11.29 and 11.48 for propranolol. Again the urapidil dose ratios did not differ significantly from 1 while the propranolol dose ratios were clearly higher.

The average duration of the linear segment of the propranolol response curves was 50.5 min. Plasma concentration of propranolol concentration fell, on average, 16% over this period. In multiple linear regression analysis, however, the propranolol concentration term reached significance at the 5% level in only one subject. That is, in all but one of ten subjects, change in propranolol concentration during the study did not contribute significantly to the slope of the dose response curves.

Two subjects complained of nasal congestion during and for several minutes after the 30 mg urapidil infusion. In one subject, symptomatic orthostatic hypotension persisted for approximately 3 h after urapidil 30 mg. No other adverse effects were noted.

Discussion and Conclusions

This study was designed to compare the $beta_1$-blocking actions of a moderate (15 mg) and a relatively high intravenous dose of urapidil (30 mg) and a relatively low intravenous dose of propranolol (5 mg). Our study has shown no significant beta blocking activity of urapidil at these doses but we demonstrated significant beta blockade after propranolol in all but one subject. The 30 mg dose came very near to causing a statistically significant degree of beta blockade as measured by this method ($p = 0.06$). We would interpret this as representing a true pharmacological effect rather than simply a chance occurrence. (That is, urapidil does appear to exhibit very weak beta blocking activity in high doses). The degree of beta blockade achieved was, however, minimal compared to that seen with propranolol 5 mg. It is unlikely therefore, that this degree of beta blockade is clinically significant. The plasma concentrations achieved after single doses of 30 mg urapidil in this study are, however, similar to those already shown to have clinically useful antihypertensive effect in chronic oral studies [15]. Mean plasma urapidil concentrations of 580 ng/ml were attained with chronic oral dosing at 60 mg twice daily (Chiariello, personal communication) compared with mean plasma concentrations of 703 ng/ml at the start and 476 ng/ml at the end of the urapidil 30 mg phase in this study. Thus, the single intravenous doses used in our study produced plasma concentrations approximately equivalent to the 30-60 mg twice daily oral doses sufficient to control most cases of mild and moderate hypertension in a recent multicenter study [15].

The effective dose range of urapidil in congestive heart failure is not yet known. We have, however, shown 15 mg urapidil iv to have significant alpha blocking activity (Jamieson, personal communication) which suggests that clinically useful doses in heart failure may be less than those required in hypertension.

In constructing dose response curves we have used a relatively high number of isoproterenol doses, compared for example to the earliest dose response studies [16, 17] which typically used 4 or 5 doses. The higher number of doses should allow more confidence in the 'best fit' terminal linear segment, particularly when a number of points are obtained around the "threshold" of drug effect. However, declining plasma drug concentrations over a prolonged dose response study might be expected to steepen the slope of the terminal segment particularly when a close relationship exists between plasma concentration and degree of beta blockade. In this study, propranolol concentration did not contribute signifi-

cantly to the regression equations nor was the response to a given dose of isoproterenol significantly influenced by propranolol concentrations. We therefore conclude that waning propranolol concentration was not reflected in decreasing beta blockade over the course of the dose responses.

Earlier studies using higher doses of propranolol and achieving dose ratios greater than these have shown a close linear relationship between degree of beta blockade (expressed as dose ratio - 1) and the logarithm of plasma propranolol concentration [18]. Our data do not show such a relationship, although this may merely reflect the smaller doses of propranolol given and the smaller range of plasma propranolol concentrations achieved in this study.

Our method of analyzing the incomplete isoproterenol dose response curve has not been previously described in any form. The empirical use of a quadratic function in describing dose response curves is established [19] as is the use of ANOVA in estimating the significance of the quadratic term. The latter, for example, has recently been applied to the analysis of baroreflex sensitivity [20]. The applications of statistical hypothesis testing to the technique of serially eliminating points is new. This method provides an objective means of defining the linear segment of a dose response curve compared to the alternative of subjective visual estimation. A variety of linear and non-linear methods of analysis have been applied to human dose-response analysis. None is universally satisfactory, but there are advantages in assigning linear segments to a series of dose response curves: the principal of which are the ability to quantitate the degree of shift of the relationship and to determine whether that shift is parallel or not without the use of complex computer software.

It is unlikely that all types of dose response curves will lend themselves to analysis by our method. In particular, the quadratic term is likely to lose significance unduly early when there is marked variability in response at the low end of the response curve. In contrast the quadratic term is likely to remain significant where a shallow response curve comprises a small number of points (that is, where the curve is well described by a quadratic function throughout its length). Under these circumstances the method of Sumner et al. [19] is more appropriate. Our method appears particularly suited to the analysis of curves constructed from a relatively large number of doses, where points lying on the early non-linear part of the curves can be discarded with little effect on the power of statistical comparisons.

In conclusion, urapidil does not have significant beta blocking activity in man when given intravenously at the doses of 15 mg and 30 mg. Accordingly, these findings do not preclude further investigation of its use in the management of congestive heart failure.

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