Antihypertensive and β-adrenoceptor antagonist action of timolol

After a 4-wk placebo period, 20 patients with essential hypertension received incremental doses of timolol (20, 40, and 60 mg daily) during three consecutive 3-wk treatment periods. Throughout the study, home blood pressures (BP) were obtained twice daily. At the end of each study period, plasma renin activity (PRA), the maximum heart rate (HR) response to isoproterenol (30 ng/kg/min) and to ergometric exercise (750 to 1,050 kgm/min), and serum timolol levels were measured. Average weekly home BP of 150/100 on placebo fell to 139/93 on 20 mg of timolol ($p < 0.01$). An additional fall in diastolic BP (4 mm Hg, $p < 0.01$) occurred as the dose was increased to 60 mg. PRA, 2.6 ng/ml/hr on placebo decreased to 1.2 on 20 mg of timolol ($p < 0.05$); thereafter it did not change. The mean maximum heart rates in response to isoproterenol and exercise were 117 and 158 beats/min on placebo; the maximum rates were 64 and 112 when the patients were receiving 20 mg of timolol ($p < 0.001$). On 60 mg, mean maximum heart rates decreased to 57 with isoproterenol and to 104 with exercise ($p < 0.02$). Timolol, 20, 40, and 60 mg daily, resulted in a wide range but increasing serum concentrations of the drug. The antihypertensive effect of timolol did not correlate with the fall in PRA. PRA and the maximum HR response to isoproterenol on placebo did not predict the BP response to timolol. That PRA was not predictive of the BP response cannot be taken as conclusive because of small number of low renin patients (3) in our series.

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Timolol maleate, a beta adrenoceptor antagonist, has been shown to be an effective antihypertensive agent.[1, 6, 10, 11] It is a pure, nonselective adrenoceptor antagonist without membrane-stablizing or sympathomimetic activity. On a weight for weight basis, it is 8 to 10 times as potent as propranolol. The average effective daily antihypertensive dosage of the drug is 21 mg with a range of 15 to 30 mg.

In our study, patients with mild or moderate essential hypertension were given timolol alone as the only drug for their hypertension. To determine the antihypertensive efficacy of increasing doses of timolol, a fixed dose schedule of 20, 40, and 60 mg daily during 3 consecutive 3-wk periods was followed. Home blood pressures were obtained to establish the onset of the antihypertensive action. The antihyperten-

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sive response was related to the degree of beta inhibition and the changes in plasma renin activity. The initial heart rate and the heart rate response to endogenous and exogenous sympathetic stimulation and the renin status of patients were evaluated as predictors of the therapeutic response.

Methods

The subjects were 20 male patients, mean age, 39 (range, 23 to 56), with mild or moderate essential hypertension. Six of the subjects were black, and 14 white. The average known duration of hypertension was 7.4 yr (range, 4 mo to 25 yr). Secondary causes of hypertension were excluded by clinical examination, rapid-sequence intravenous pyelography, and laboratory studies (serum creatinine and potassium, urinanalysis, and urinary epinephrine and norepinephrine). None of the subjects had coronary heart disease, valvular heart disease, a history of cerebrovascular disease, a history of congestive heart failure, bundle branch block, atrioventricular block, chronic obstructive lung disease, a history of bronchial asthma, allergic rhinitis, renal insufficiency (serum creatinine above 1.9 mg/dl) or grade III or IV hypertensive retinopathy.

All antihypertensive medications were discontinued, and all subjects were given placebo, one tablet twice daily for 4 wk, followed by 3 consecutive 3-wk periods of incremental doses of timolol, 20, 40, and 60 mg daily, in two divided doses. Casual sitting home blood pressures, twice daily, were obtained and averaged weekly throughout the study. The subjects were seen at the end of the placebo period and at the end of each 3-wk treatment period, when the following measurements were made:

Vital signs. Clinic sitting blood pressures and heart rates were taken by the same nurse throughout the study.

Plasma renin activity (PRA). Blood for PRA determination was drawn between 9 to 11 A.M. after the subjects were standing and walking for 1 hr. PRA was determined by radioimmunoassay of angiotensin I according to the method of Haber and co-workers.[8] PRA values were related to the urinary sodium excretion during the 24-hr period that preceded the drawing of blood.[5]

Heart rate response to isoproterenol infusion. After 10 min of supine rest, isoproterenol, 30 ng base/kg/min, was administered for 12 min into an antecubital vein from a constant-infusion pump. The heart rate response to isoproterenol was recorded electrocardiographically, and the maximum heart rate achieved during the infusion was obtained.

Heart rate response to ergometric exercise. For each subject, the ergometer was set at a constant load, which was shown prior to the study to produce a heart rate of at least 150 beats/min after 2-min exercise. The average load in the 20 subjects was 960 kgm/min (range, 750 to 1,050). The maximum heart rate at the end of 2-min exercise was obtained from a 15-sec strip of the electrocardiogram.

Serum timolol concentrations. In 15 subjects, blood for serum timolol levels was obtained at the end of each 3-wk treatment period. Blood was drawn between 9 and 11 A.M., 2 to 4 hrs after the morning dose of timolol. The serum was separated by centrifugation and stored at −21° C before being sent for analysis. Serum timolol concentration was measured by the electron-capture gas chromatographic method.*

Student's t test, the t test for paired observations and correlation coefficients were calculated by means of the Midas (Constat) computer program of the Statistical Research Laboratory at the University of Michigan.

Results

The average weekly home blood pressures for the last week of each study period and the clinic blood pressures are listed in Table I. Average weekly home and clinic blood pressures, 150/100 and 151/102 on placebo, fell to 139/93 and 135/94 on 20 mg of timolol ($p < 0.01$). An additional fall in the average home diastolic blood pressures occurred in every subject during the next 6 wk as the dose of timolol was increased to 40 and then to 60 mg. Average clinic blood pressures remained unchanged as

*Courtesy of Merck, Sharp & Dohme Research Laboratories.

Table I. *Mean (±SEM) weekly home* and clinic blood pressures of 20 hypertensive subjects during placebo and timolol treatment*

Blood pressure (mm Hg)	*Placebo*	*p value (placebo vs 20 mg)*	*Timolol (mg)*			*p value (20 vs 60 mg)*
			20	*40*	*60*	
Home	150 ± 2.9	<0.001	139 ± 2.4	136 ± 2.7	136 ± 3.1	NS
	100 ± 2.0	<0.01	93 ± 2.1	89 ± 2.1	89 ± 2.4	<0.01
Clinic	151 ± 4.6	<0.001	135 ± 3.7	136 ± 4.4	137 ± 3.6	NS
	102 ± 2.6	<0.01	94 ± 2.4	93 ± 2.9	94 ± 2.7	NS

*Mean weekly home blood pressure during the last week of each study period is shown.

Table II. *Mean (±SEM) weekly blood pressures of 14 responders* to the antihypertensive effect of timolol*

Blood pressure (mm Hg)	*Placebo*	*p value (placebo vs 20 mg)*	*Timolol (mg)*			*p value (20 vs 60 mg)*
			20	*40*	*60*	
Home	148 ± 4.0	<0.001	134 ± 2.4	132 ± 3.2	130 ± 2.5	<0.05
	100 ± 2.2	<0.001	90 ± 2.3	87 ± 2.7	86 ± 2.4	<0.01

*Defined as ≥10 mm Hg fall in mean arterial blood pressure at home on 60 mg of timolol compared with values on placebo.

the dose of timolol was increased. In 14 subjects, defined as responders, mean arterial blood pressures at home were reduced by 10 mm Hg or more at the end of the study. The pattern of home blood pressure responses to increasing doses of timolol in responders was similar to that of the group as a whole, except that a small additional decrease also occurred in systolic blood pressures as the dose of timolol was increased from 20 to 60 mg (Table II).

In 14 subjects, the records of the twice-daily home blood pressures were sufficiently complete to allow a day-by-day analysis of the blood pressure response to 20 mg of timolol. The average home blood pressure at the end of the placebo period, 150/104, fell to 142/96 ($p < 0.05$) by the end of the first day and to 139/95 ($p < 0.01$) by the end of the second day of treatment. The average home blood pressure by the end of the first week of therapy with 20 mg of timolol was 136/92 ($p < 0.001$). There was no additional antihypertensive effect of 20 mg of timolol in these subjects during the remaining 2 wk of treatment.

Average clinic sitting and isoproterenol- and exercise-induced heart rates are summarized in Table III. Sitting heart rates, 74 ± 1.9 (mean ± SEM) on placebo, were reduced to 63 ± 1.6 on 20 mg of timolol ($p < 0.001$). There was no further decrease in sitting heart rate as the dose of timolol was increased. The mean maximum heart rates in response to a fixed dose of isoproterenol and to a fixed exercise load, 117 and 158 beats/min (mean) on placebo, were reduced to 64 and 112 on 20 mg of timolol ($p < 0.001$). On 60 mg of timolol, heart rate decreased to 57 on isoproterenol and to 105 on exercise ($p < 0.02$).

Based on a nomogram, derived in our laboratories, relating plasma renin activity (PRA) to 24-hr urinary excretion, PRA on placebo was low in 3, normal in 12, and high in 5 subjects.[8] The plasma renin activities of our subjects, 2.6 ± 0.51 ng/ml/min (mean ± SEM, N = 20) on placebo, was reduced ($p < 0.05$) to 1.2 ± 0.25, 1.4 ± 0.31, and 1.3 ± 0.24, respectively, on 20, 40 and 60 mg of timolol.

In 15 subjects, mean ± SEM serum timolol levels (ng/ml) on 20, 40, and 60 mg of timolol were: 42 ± 7.0 (range, 0 to 86), 77 ± 10.8 (range, 30 to 156), and 96 ± 12.6 (range, 39 to 171). Five of the 15 subjects were nonresponders to the antihypertensive effect of timolol. Serum timolol levels on 20, 40, and 60 mg of the drug, 36, 82, and 109 ng/ml (mean), were similar to those of the group as a whole. With one exception, a high degree of beta adrenergic inhibition was achieved with 20 mg of timolol

Table III. *Mean (±SEM) sitting and isoproterenol and exercise-induced heart rate of 20 hypertensive subjects on placebo and 20, 40, and 60 mg of timolol*

Heart rate (beats/min)	*Placebo*	*p value (placebo vs 20 mg)*	*Timolol (mg)*			*p value (20 vs 60 mg)*
			20	*40*	*60*	
Sitting	74 ± 1.9	<0.001	63 ± 1.6	63 ± 1.8	61 ± 1.3	NS
Isoproterenol*†	117 ± 3.8	<0.001	64 ± 2.7	60 ± 1.2	57 ± 1.2	<0.02
Exercise*	158 ± 2.3	<0.001	112 ± 2.2	107 ± 2.1	105 ± 2.0	<0.02

*Mean maximum heart rates obtained in response to isoproterenol and exercise are shown.

†Mean supine heart rates before isoproterenol infusion were: 73 ± 2.5, 59 ± 1.5, 58 ± 1.3, and 56 ± 1.3 on placebo and 20, 40, and 60 mg of timolol.

Table IV. *Comparisons of mean (±SEM) weekly home blood pressures, sitting and isoproterenol- and exercise-induced heart rates, and plasma renin activities of responders (N = 14) and of nonresponders (N = 6) on placebo and on 20 mg of timolol*

Parameter	*Placebo*		*Timolol (20 mg)*	
	Responders	*Nonresponders*	*Responders*	*Nonresponders*
Home blood pressure (mm Hg)	148 ± 4.0 / 100 ± 2.2	153 ± 3.2 / 99 ± 4.3	134 ± 2.4 / 90 ± 2.3	149 ± 3.1* / 99 ± 4.0†
Heart rate (beats/min)				
Sitting	75 ± 2.1	72 ± 4.2	63 ± 2.1	64 ± 2.4
Isoproterenol‡	117 ± 3.6	116 ± 10.4	62 ± 1.7	69 ± 8.2
Exercise‡	158 ± 2.5	156 ± 5.2	111 ± 2.7	114 ± 4.4
Plasma renin activity (ng/ml/hr)	3.0 ± 0.63	1.4 ± 0.47	1.5 ± 0.31	0.7 ± 0.15

*$p < 0.01$ for comparisons of values in responders and nonresponders.

†$p < 0.05$ for comparisons of values in responders and nonresponders.

‡Mean maximum heart rates obtained in response to isoproterenol and exercise are shown.

in the 15 subjects despite variations in serum timolol levels. In the only subject with an unmeasurable serum timolol level, the heart rate rose to 108 beats/min with isoproterenol and to 126 with exercise. There were no statistically significant correlations between the serum concentration of drug and degree of beta adrenergic inhibition.

Finally, the home blood pressure, the heart rate, and the PRA responses to 20 mg of timolol of responders and of nonresponders were compared (Table IV). At the end of placebo period, the two groups were comparable with regard to blood pressure and heart rate responses. The numeric difference in the average PRA of responders and of nonresponders during the placebo period did not achieve statistical significance ($p > 0.1$). During treatment with 20 mg of timolol, there was a similar reduction in sitting and isoproterenol- and exercise-induced heart rates and in PRA in responders and nonresponders. The change in mean arterial blood pressure between placebo and 20 mg of timolol was unrelated to the change in PRA in both the entire group of subjects ($r = 0.14$, $N = 20$) and in responders ($r = 0.09$, $N = 14$). There was no reduction in blood pressure by 20 mg of timolol in all 3 low renin hypertensive subjects. When the dose of timolol was raised to 60 mg, 2 of the low renin hypertensive subjects responded.

Discussion

In this group of patients with mild to moderate essential hypertension, the principal antihypertensive effect of timolol, 73% of the total reduction in the average mean arterial blood pressure, occurred with 20 mg of timolol daily, the lowest dose schedule. Further analysis of the home blood pressures showed that the reduction in mean arterial blood pressure due to 20 mg of timolol was complete

at the end of the first week and that there had been a significant reduction in blood pressure by the end of the first day. It appears that the hemodynamic adjustments that account for the antihypertensive effect of timolol are complete within the first few days of the administration of an effective beta inhibitor dose. The small additional antihypertensive effect of 40 and 60 mg of timolol seems to be related to the enlargement of the dose rather than to the long-term hemodynamic adjustments to the initial 20-mg dose.

During treatment with 20 mg of timolol, clinic sitting heart rates and plasma renin activities reached their lowest level. The heart rate response to a fixed dose of isoproterenol and to a fixed load of exercise was reduced 88% and 87% of the maximum reduction achieved with 60 mg of timolol. A small additional beta adrenoceptor–inhibiting effect of timolol was noted as the dose was increased to 40 and 60 mg daily. This finding accords with the receptor theory of drug antagonism. Similar degrees of beta inhibition were achieved in the blood pressure responders and the nonresponders by the three dose levels used. Beta adrenoceptor inhibition, therefore, is not the only determinant of the antihypertensive action of timolol.

A theory of dual antihypertensive action of beta adrenoceptor antagonists has been proposed.[9] According to this theory, the first and principal antihypertensive effect occurs with low serum concentrations and is associated with the drug's renin-lowering properties. The second effect requires higher serum concentrations and is not related to an additional lowering of plasma renin activity. The findings of this study support the observation of an additional antihypertensive effect of the higher doses of a beta adrenoceptor antagonist drug.

There was no correlation between the antihypertensive and the renin-lowering effect of 20 mg of timolol, but the study was not designed to investigate such a relationship. The initial dose of timolol was probably in excess of that required to suppress renin, resulting perhaps in an antihypertensive effect unrelated to the reduction in plasma renin activity. The number of patients (3 in all) with low renin hypertension was small.

It has been suggested that hypertensive patients with a hyperkinetic circulation, characterized in part by an increased resting heart rate and an increased cardioaccelerator response to the intravenous infusion of a beta adrenoceptor agonist, are more likely to respond with a reduction in blood pressure to a beta adrenoceptor antagonist drug.[7] Bühler and co-workers[2] have reported that hypertensive patients with high PRA exhibit favorable blood pressure responses to low to moderate doses of propranolol, whereas patients with low renin hypertension exhibit a negligible blood pressure response. In our study,[2] the resting heart rate and the heart rate response to isoproterenol while our patients were on placebo were of no value in predicting the blood pressure response to timolol. At the end of the placebo period, the PRA of responders was twice that of nonresponders but the difference was not statistically significant. Had we enlarged our series and included more patients with low renin hypertension, the difference in baseline PRA of responders and nonresponders might have reached statistical significance and confirmed the reports of Bühler and colleagues,[2] and of others.[9]

Timolol, 20, 40, and 60 mg, resulted in a wide range of serum concentrations of the drug, but the average values increased with each increment in dosage. The wide range of serum concentrations can be accounted for in part by the variable time interval (2 to 4 hr) between the morning dose of the drug and the drawing of blood for the serum concentration measurements. Serum concentrations of the drug did not seem to bear a relationship to either the degree of beta adrenoceptor inhibition or the antihypertensive effect of the drug. The wide range of serum timolol levels and the high degree of beta adrenergic inhibition achieved with 20 mg of timolol were probably responsible for the lack of correlation between the drug levels and the degrees of beta adrenergic inhibition. A more complete study of serum concentrations, which would permit calculation of the area under the time-concentration curve, might have revealed a correlation between the serum concentrations of drug and pharmacologic effect. There were no data in the literature with which to compare our values of serum timolol concentrations.

The design of this study evoked a comparison

of beta inhibition by isoproterenol infusion and by exercise. Exercise, an endogenous sympathetic stimulus, inducing heart rate responses above 130 beats/min during a placebo period, has been the preferred test of the degree of beta inhibition achieved with beta adrenoceptor antagonists.[3] Isoproterenol-induced tachycardia, an exogenous sympathetic stimulus, also has been used and is most accurate when a dose-response curve is established.[4] In our study, no attempt was made to establish a dose-response curve for exogenous beta stimulation. The results obtained with isoproterenol were similar to those achieved with exercise testing at all three dose levels of timolol.

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