Abstract

Background: Endothelin is a powerful vasoconstrictor peptide derived from the endothelium. We evaluated the contribution of endothelin to blood-pressure regulation in patients with essential hypertension by studying the effect of an endothelin-receptor antagonist, bosentan.

Methods: We studied 293 patients with mild-to-moderate essential hypertension. After a placebo run-in period of four to six weeks, patients were randomly assigned to receive one of four oral doses of bosentan (100, 500, or 1000 mg once daily or 1000 mg twice daily), placebo, or the angiotensin-converting-enzyme inhibitor enalapril (20 mg once daily) for four weeks. Blood pressure was measured before and after treatment.

Results: As compared with placebo, bosentan resulted in a significant reduction in diastolic pressure with a daily dose of 500 or 2000 mg (an absolute reduction of 5.7 mm Hg at each dose), which was similar to the reduction with enalapril (5.8 mm Hg). There were no significant changes in heart rate. Bosentan did not result in activation of the sympathetic nervous system (as determined by measurement of the plasma norepinephrine level) or the renin-angiotensin system (as determined by measurements of plasma renin activity and angiotensin II levels).

Conclusions: An endothelin-receptor antagonist, bosentan, significantly lowered blood pressure in patients with essential hypertension, suggesting that endothelin may contribute to elevated blood pressure in such patients. The favorable effect of treatment with bosentan on blood pressure occurred without reflexive neurohormonal activation. (N Engl J Med 1998;338:784-90.)

Endothelin-1 is a potent endothelium-derived vasoconstrictor peptide that has been implicated in the pathogenesis of hypertension and chronic heart failure. [1] Plasma levels of endothelin-1 have been found to be elevated in some but not all studies of patients with essential hypertension. [2,3] Furthermore, administration of specific endothelin-receptor antagonists has resulted in reductions in blood pressure in certain animal models of hypertension, [4,5] suggesting that endothelin-1 has a role in blood-pressure elevation. However, the effect of long-term endothelin-receptor antagonism on blood-pressure control in patients with essential hypertension has not been determined.

Nonpeptidergic, orally active endothelin-receptor antagonists have been developed, [4,6] permitting long-term administration. Two types of endothelin receptors have been described: ET_A) and ET_B) receptors. [7,8] Both types have been identified on vascular smooth-muscle cells and found to mediate vasoconstriction, [9] whereas only the ET_B) receptor has been identified on endothelial cells. Activation of the endothelial-cell ET_B) receptor mediates vasodilatation when exogenous endothelin is administered [10]; therefore, ET_B) receptors can mediate both constriction and dilatation.

Mixed ET_A))-receptor and ET_B))-receptor antagonists as well as selective ET_A))-receptor antagonists have been developed, permitting an assessment of the contribution of endothelin-1 to various cardiovascular diseases. Bosentan is a highly specific, orally active mixed ET_A))-receptor and ET_B))-receptor antagonist suitable for long-term administration. [6] Administration of bosentan in animals has clearly been associated with reductions in blood pressure, [11] suggesting that the overall effect of antagonism with mixed endothelin receptors is vasodilatation.

We performed a study to determine whether endothelin-1 contributes to elevated blood pressure in patients with essential hypertension by assessing the effect of four weeks of treatment with bosentan on blood pressure and heart rate. In addition, the effect of endothelin-receptor antagonism with bosentan on cardiovascular neurohormonal status was examined.

Methods

Study Population

Men and women 18 years of age or older were enrolled in the study if they had essential hypertension, defined as an average mean diastolic pressure of 95 to 115 mm Hg after a four-week run-in period with placebo. To make sure the patients had stable blood pressure, the diastolic pressure, measured while the patient was sitting upright, could not differ by more than 7 mm Hg on three consecutive visits. In addition, patients were required to have a mean diastolic pressure higher than 85 mm Hg on 24-hour ambulatory blood-pressure monitoring.

The study was approved by the institutional review board at each participating center, and all patients provided written informed consent before the start of the study.

Study Procedures

All patients eligible for the study underwent screening (which included a period of tapering and discontinuation of current antihypertensive therapy), followed by a placebo run-in period of four to six weeks. At the end of the run-in period, base-line measurements of blood pressure and heart rate, determined in the office and with ambulatory monitoring, as well as serum creatinine levels, were performed.

Office measurement of systolic and diastolic pressure was performed manually with a calibrated mercury sphygmomanometer and the use of the American Heart Association criteria. The patient was required to remain in a sitting position for at least five minutes, after which two blood-pressure measurements separated by one minute were made. Heart rate was measured manually at the same time as blood pressure. These assessments were made immediately before the administration of the study drug in order to match subsequent assessments of blood pressure and heart rate at trough levels of the study drug - that is, 12 hours after the dose for twice-daily administration of bosentan (in the group receiving a total daily dose of 2000 mg) and 24 hours after the dose for once-daily administration of bosentan and enalapril.

Twenty-four-hour ambulatory blood-pressure monitoring was performed with a portable recording system (Spacelabs 90207, Spacelabs Medical, Redmond, Wash.). Measurements included mean 24-hour diastolic and systolic pressure, as well as daytime values (measured every 15 minutes from 7 a.m. to 10 p.m.) and nighttime values (measured every 20 minutes from 10 p.m. to 7 a.m.).

A subgroup of patients underwent evaluation of relevant neurohormonal factors. Plasma for neurohormonal measurements was collected in a standardized manner among the study sites. Blood specimens were obtained when the patients had been in the sitting position for at least 30 minutes after the insertion of an intravenous cannula in the antecubital fossa. Blood was collected in chilled polypropylene EDTA tubes, spun in a cold (4 degreesC) centrifuge for 10 minutes at 2000 revolutions per minute (except that specimens were centrifuged at ambient temperature for measurement of plasma renin activity), then immediately placed in a -70 degreesC freezer. Batched samples were shipped on dry ice to a central laboratory (Medi-Lab, Copenhagen, Denmark) for analysis on receipt.

The neurohormonal factors measured were plasma norepinephrine (an index of sympathetic activity), plasma renin activity, plasma angiotensin II (an index of renin-angiotensin activity), plasma endothelin-1, and plasma big endothelin-1 (the biologic precursor of active endothelin-1).

Plasma angiotensin II was measured by radioimmunoassay according to the method of Kappelgaard et al. [12] Plasma levels of big endothelin-1 and endothelin-1 were measured by radioimmunoassay according to the methods of Loffler and Maire. [13] Plasma renin activity and plasma catecholamines are routinely measured in the laboratory with the use of validated techniques of radioimmunoassay and high-performance liquid chromatography, respectively. Normal ranges are as follows: plasma renin activity, 10.5 to 77 mIU per liter in the upright position and 8.8 to 36 mIU per liter in the supine position; plasma angiotensin II, 3.8 to 30 ng per liter in the supine position; endothelin-1, 2.0 to 4.6 ng per liter; plasma big endothelin-1, 7.8 to 16.4 ng per liter; and plasma norepinephrine, 0.66 to 3.85 nmol per liter. The coefficient of variation was less than 15 percent for all assays.

After the base-line evaluations had been performed, the patients were randomly assigned in a double-blind manner to receive four weeks of therapy with one of six treatments: placebo; bosentan at a dose of 100, 500, or 1000 mg once daily or 1000 mg twice daily; or the angiotensin-converting-enzyme inhibitor enalapril (20 mg once daily). After the four-week double-blind study period, the patients again underwent the evaluations that had been performed at base line.

Statistical Analysis

The primary end point of the study was the change from base line to week 4 in diastolic pressure measured in the office with the patient sitting upright. Secondary end points were changes from base line in systolic pressure measured in the office and diastolic and systolic pressure on ambulatory monitoring, as well as changes from base line in neurohormonal measurements.

We analyzed the primary end point by testing the dose-response relation for bosentan (a trend test for placebo and bosentan at doses of 100, 500, 1000, and 2000 mg), with pairwise comparisons of placebo and bosentan doses when the result of the overall trend test was significant. Tests were performed with an analysis-of-variance model and normal-distribution approximations. [14] All P values are two-tailed and adjusted for each variable for multiple comparisons according to the closed-test principle of many-to-one comparisons. [15] A two-tailed P value of less than 0.05 was considered to indicate statistical significance.

Twenty-six patients were excluded from the analysis because they did not undergo blood-pressure measurements at week 4: 4 patients receiving placebo; 18 receiving bosentan (6 in the 100-mg group, 4 in the 500-mg group, 2 in the 1000-mg group, and 6 in the 2000-mg group); and 4 receiving enalapril.

For the neurohormonal data, some values differed from the mean by more than 5 SD. Whether these values were accurate or were the result of laboratory errors could not be determined. Therefore, these data were analyzed nonparametrically with the use of the Kruskal-Wallis test.

We calculated the power of the study on the basis of a total sample of 300 patients (50 in each group) during active treatment, with the expectation that dropouts and protocol violations would result in a total of 240 patients (40 in each group) who could be evaluated at the completion of the study. The study had a power of more than 90 percent to detect a linear trend and a power of at least 80 percent to detect differences of 6 mm Hg or more in diastolic pressure in the sitting position between the bosentan groups and the placebo group, with a two-tailed significance level of 5 percent, assuming a standard deviation of 7 mm Hg for the change from the base-line diastolic pressure.

Results

Characteristics of the Patients

Of the 511 patients screened for the study, 293 met the criteria for double-blind randomization to one of the six treatment groups. The six groups were well balanced with regard to age, sex, weight, height, race, systolic and diastolic pressure in the sitting position, heart rate, serum creatinine levels, blood pressure on ambulatory monitoring, and plasma levels of neurohormonal factors (Table 1).

CHARACTERISTIC	PLACEBO (N=49)	BOSENTAN				ENALAPRIL (N=50)
		100 mg (N=50)	500 mg (N=49)	1000 mg (N=45)	2000 mg (N=50)	
		mean ±SD				
Male sex (%)	55	70	76	64	74	66
Age (yr)	56±9	56±9	55±10	54±10	55±11	59±10
Weight (kg)	76±13	82±11	83±11	83±13	85±14	79±13
Height (cm)	168±11	171±9	173±8	171±8	173±8	171±8
Heart rate (bpm)	71.8±7.8	73.7±7.5	73.5±8.1	74.8±7.5	73.4±7.7	76.2±8.4
Office blood-pressure measurements (mm Hg)						
Diastolic pressure	101.7±4.5	103.9±4.1	101.1±5.0	107.4±4.7	102.9±4.8	102.2±5.0
Systolic pressure	158.3±14.1	168.6±16.3	161.2±14.3	161.5±14.7	161.3±13.2	161.9±14.3
Ambulatory blood-pressure monitoring (mm Hg)						
24-Hr diastolic pressure	94.4±7.2	97.6±6.7	94.1±6.7	96.4±6.5	96.2±7.5	94.1±5.3
Daytime diastolic pressure	99.2±7.1	102.6±7.3	98.5±7.6	100.8±6.1	101.0±7.4	99.2±5.7
Nighttime diastolic pressure	85.2±8.9	87.9±7.6	85.5±8.1	87.7±8.4	86.6±9.4	83.5±7.0
24-Hr systolic pressure	149.5±13.4	160.4±16.9	153.7±12.3	152.6±14.0	153.6±11.6	152.3±12.0
Daytime systolic pressure	155.2±13.7	166.8±18.0	158.9±12.8	155.4±14.0	158.2±11.9	158.6±12.3
Nighttime systolic pressure	138.4±15.2	147.8±17.2	143.3±14.2	142.0±15.6	142.0±13.5	138.6±14.6
Serum creatinine (μmol/liter)†	90±14	93±15	94±17	94±11	92±13	91±15
Neurohormonal factors‡						
Plasma norepinephrine (nmol/liter)	1.8±0.9	1.4±1.1	1.5±1.0	1.7±0.9	1.8±1.3	1.9±1.0
Plasma renin activity (mIU/liter)	18.4±10.4	17.8±11.0	25.4±30.9	27.0±25.0	15.2±8.9	16.3±12.6
Plasma angiotensin II (ng/liter)	8.2±10.0	11.7±21.8	12.1±23.7	8.0±9.3	5.6±6.0	9.9±17.4
Plasma endothelin-1 (ng/liter)	4.1±0.5	4.0±0.8	4.2±1.1	4.0±0.7	4.0±1.0	4.0±0.9
Plasma big endothelin-1 (ng/liter)	13.3±2.1	13.1±3.3	14.4±4.4	13.4±2.6	13.3±2.5	13.7±3.3

*All measurements were made immediately before the start of therapy. Office measurements of blood pressure were made while the patients were sitting upright. Patients assigned to receive bosentan were given 100, 500, or 1000 mg once a day or 1000 mg twice a day (the 2000-mg group). Patients assigned to receive enalapril were given 20 mg once a day.

†To convert the values for creatinine to milligrams per deciliter, divide by 88.4.

‡Normal ranges are as follows: norepinephrine, 0.66 to 3.85 nmol per liter; plasma renin activity: upright, 10.5 to 77 mIU per liter, and supine, 8.8 to 36 mIU per liter; angiotensin II: supine, 3.8 to 30 ng per liter; endothelin-1, 2.0 to 4.6 ng per liter; and big endothelin-1, 7.8 to 16.4 ng per liter. Values more than 5 SD from the mean are excluded from the table but were included in the nonparametric analysis. There were two such outlying values, both in the group of patients receiving 500 mg of bosentan daily: angiotensin II, 173.0 ng per liter; and plasma renin activity, 900.0 mIU per liter. To convert the values for norepinephrine to picograms per milliliter, divide by 0.005911.

Table 1. Base-Line Characteristics of the Patients.

Eleven patients withdrew from the study before its completion because of adverse events. Two patients had myocardial infarctions (one was receiving enalapril, and the other was receiving 100 mg of bosentan daily). Six patients receiving bosentan - four in the 2000-mg group, one in the 500-mg group, and one in the 100-mg group - withdrew because of headache, edema, or flushing. Three patients in the placebo group withdrew, one each because of joint pains, chest pains,

and headache.

All patients randomly assigned to a treatment group completed at least one week of oral therapy. The mean duration of active treatment was similar in all six groups, ranging from 25.9 to 27.6 days. A total of 267 patients had completed the week 4 assessment at the time the study was closed for analysis of adverse effects and efficacy.

Office Blood-Pressure Measurements

The primary end point of the study (the change from base line in the office measurement of diastolic pressure in the sitting position) is shown in Figure 1 for each of the six groups. The effect of bosentan, as compared with placebo, in reducing diastolic pressure was significant (P = 0.02 by the trend test), and pairwise comparisons were significant for the 500-mg and 2000-mg daily doses. The effect of bosentan on blood pressure at these doses was similar to that of enalapril given in a daily dose of 20 mg.

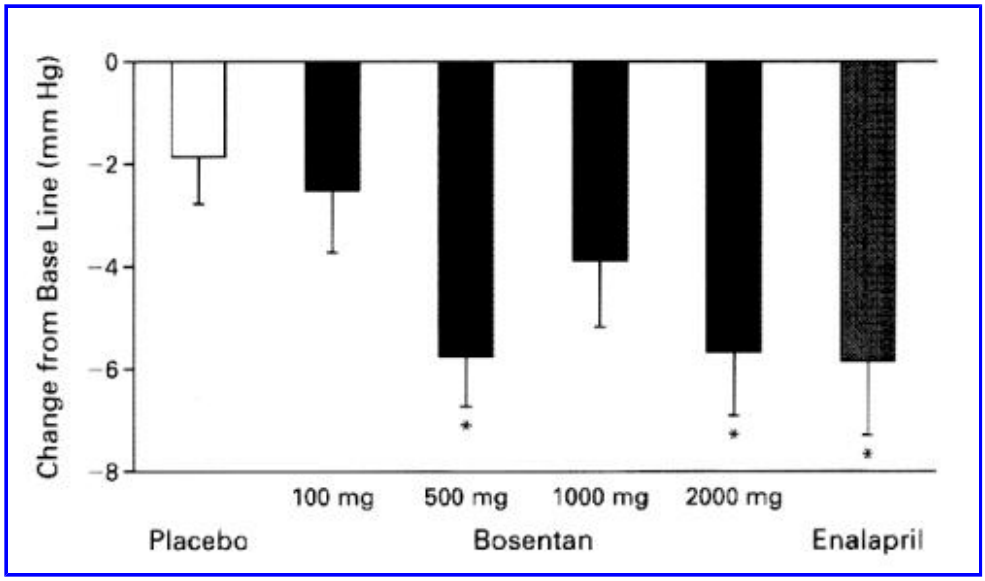

Figure 1. Mean (+/-SE) Change from Base Line in Diastolic Pressure in Patients with Mild-to-Moderate Hypertension Assigned to Receive Placebo, Bosentan (100, 500, 1000, or 2000 mg Daily), or Enalapril (20 mg Daily). Measurements were made while the patients were sitting upright. Asterisks denote P<0.05 for the comparison with placebo. P = 0.02 by the trend test for the comparison of the four doses of bosentan.

The change in the office measurement of systolic pressure in the sitting position, a secondary end point of the study, is shown in Figure 2. The effect of bosentan, as compared with placebo, in reducing systolic pressure was significant (P = 0.001 by the trend test), and pairwise comparisons were significant at the three highest doses of bosentan (500, 1000, and 2000 mg). Again, the magnitude of the reduction in blood pressure at these doses was similar to that with enalapril.

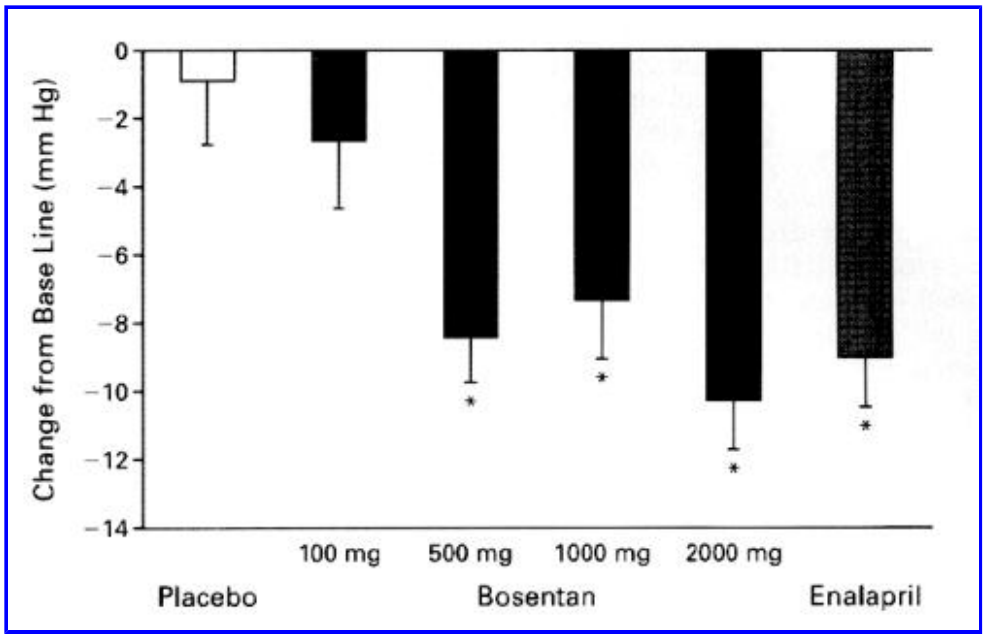

Figure 2. Mean (+/-SE) Change from Base Line in Systolic Pressure in Patients with Mild-to-Moderate Hypertension Assigned to Receive Placebo, Bosentan (100, 500, 1000, or 2000 mg Daily), or Enalapril (20 mg Daily). Measurements were made while the patients were sitting upright. Asterisks denote P<0.05 for the comparison with placebo. P = 0.001 by the trend test for the comparison of the four doses of bosentan.

The mean (+/-SE) change in heart rate was not significantly associated with the change in blood pressure (placebo group, -0.76+/-0.98 bpm; 100-mg group, -2.67+/-1.04 bpm; 500-mg group, -2.32+/-0.97 bpm; 1000-mg group, -1.17+/-1.21 bpm; 2000-mg group, -0.02+/-0.89 bpm; and enalapril group, -1.09+/-1.18 bpm).

Ambulatory Blood-Pressure Measurements

The reductions in mean 24-hour, daytime, and nighttime diastolic pressures in the four bosentan groups were significantly larger than those in the placebo group (P = 0.001 by the trend test) (Table 2). The reduction in blood pressure was greatest in the 2000-mg group. There were no significant differences in mean changes in daytime diastolic pressure among the four bosentan groups. However, nighttime diastolic pressure was significantly lower in the 2000-mg group than in the 100-mg, 500-mg, and 1000-mg groups (P = 0.001 for all comparisons), suggesting that only the 2000-mg dose (1000 mg administered twice a day) lowers blood pressure for a full 24 hours. The findings were similar with measures of systolic pressure (Table 2). The blood-pressure reductions in the bosentan groups did not differ statistically from those in the enalapril group.

Blood Pressure	Placebo	Bosentan				Enalapril
		100 mg	500 mg	1000 mg	2000 mg	
		mean ±SE				
Office measurement (mm Hg)†						
No. of patients	*45*	*44*	*45*	*43*	*44*	*46*
Diastolic pressure	−1.8±1.0	−2.5±1.1	−5.7±1.0	−3.9±1.1	−5.7±1.1	−5.8±1.0
Systolic pressure	−0.9±1.7	−2.5±1.8	−8.4±1.7	−7.4±1.8	−10.3±1.8	−9.0±1.7
Ambulatory monitoring (mm Hg)						
No. of patients	*40*	*36*	*37*	*37*	*38*	*39*
24-Hr diastolic pressure	−0.6±0.9	−5.6±0.9	−6.8±0.8	−5.5±0.9	−8.6±0.9	−7.8±0.8
Daytime diastolic pressure	−1.0±0.9	−6.2±1.0	−7.0±0.9	−6.1±1.0	−7.9±0.9	−8.4±0.9
Nighttime diastolic pressure	−0.1±1.0	−4.6±1.1	−5.1±1.0	−4.4±1.0	−10.4±1.0	−6.0±1.0
24-Hr systolic pressure	−0.7±0.9	−6.4±1.0	−9.5±0.9	−6.7±1.0	−11.0±0.9	−12.5±0.9
Daytime systolic pressure	−1.0±1.0	−6.7±1.1	−9.4±1.0	−7.6±1.0	−9.9±1.0	−13.4±1.0
Nighttime systolic pressure	−0.1±1.0	−6.1±1.1	−8.9±1.0	−5.0±1.0	−13.5±1.0	−10.2±1.0

*P<0.05 for all comparisons with placebo except office measurements of systolic and diastolic pressure in the 100-mg group and diastolic pressure in the 1000-mg group.

†Blood pressure was measured when the study drug had reached trough levels.

Table 2. Changes from Base Line in Blood Pressure.

There were no significant correlations between age, weight, or serum creatinine level and changes in ambulatory blood pressure in response to bosentan.

Measurement of Renal Function

Base-line plasma creatinine levels were similar among the six treatment groups. Changes in the plasma creatinine level after four weeks of therapy did not differ significantly among the treatment groups and were not of clinical significance. The plasma creatinine level did not increase as compared with base-line values (mean change: placebo group, -0.02+/-0.01 mg per deciliter [-2+/-1 micromol per liter]; 100-mg group, -0.02+/-0.01 mg per deciliter [-2+/-1 micromol per liter]; 500-mg group, -0.01+/-0.01 mg per deciliter [-1+/-1 micromol per liter]; 1000-mg group, -0.03+/-0.01 mg per deciliter [-3+/-1 micromol per liter]; 2000-mg group, -0.01+/-0.01 mg per deciliter [-1+/-1 micromol per liter]; and enalapril group, 0+/-0.01 mg per deciliter [0+/-1 micromol per liter]).

Measurement of Plasma Neurohormonal Factor Levels

Changes from base line in neurohormonal measurements are summarized in Table 3. There were no significant differences in trough plasma levels of norepinephrine in patients receiving bosentan as compared with placebo (P = 1.0 by the trend test). Similarly, changes in plasma renin activity and angiotensin II levels did not differ significantly between patients receiving bosentan and those receiving placebo (P = 0.8 and P = 0.5, respectively, by the trend test). There was, however, the expected increase in plasma renin activity in patients receiving enalapril.

Neurohormonal Factor	Placebo (N=19)	Bosentan				Enalapril (N=20)
		100 mg (N=17)	500 mg (N=22)	1000 mg (N=17)	2000 mg (N=21)	
		mean ±SE				
Plasma renin activity (mIU/liter)	−2.5±6.6	1.4±7.0	−4.9±6.2	−2.7±7.0	0.7±6.3	44.1±6.5†
Angiotensin II (ng/liter)	0.3±6.3	−6.8±5.8	−2.5±5.0	−0.4±5.8	2.4±5.1	−2.8±5.3
Endothelin-1 (ng/liter)	−0.1±0.3	0.6±0.3	0.7±0.2†	0.7±0.3†	1.2±0.2†	−0.1±0.2
Big endothelin-1 (ng/liter)	−0.3±0.8	2.2±0.9†	0.9±0.8	2.0±0.9†	1.2±0.8	2.3±0.8†
Norepinephrine (nmol/liter)‡	0.3±0.3	0.4±0.3	0.5±0.3	0.6±0.3	0.2±0.3	0.3±0.3

*One value, excluded from the table but included in the nonparametric analysis, was more than 5 SD from the mean: angiotensin II, 208.0 ng per liter, in a patient receiving 500 mg of bosentan daily.

†P<0.05 for the comparison with placebo.

‡Norepinephrine was measured in 12 patients in the placebo group, 13 in the 100-mg group, 12 in the 500-mg group, 12 in the 1000-mg group, 14 in the 2000-mg group, and 15 in the enalapril group. To convert the values for norepinephrine to picograms per milliliter, divide by 0.005911.

Table 3. Changes from Base Line in Neurohormonal Factors.

There were increases in plasma levels of endothelin-1 in all groups receiving bosentan (P = 0.001 by the trend test), and the increases were significant at all doses except the lowest (100 mg daily). Although the result of the trend test for bosentan was not significant overall (P = 0.3), there were significant increases in plasma levels of big endothelin-1 in the 100-mg and 1000-mg groups as well as in the enalapril group.

There were no significant correlations between base-line plasma levels of neurohormonal factors and blood-pressure responses to bosentan. Furthermore, there were no significant correlations between changes in plasma levels of neurohormonal factors and blood-pressure responses to bosentan.

Adverse Effects

Bosentan was generally well tolerated. The incidence of reported adverse events (including those considered to be unrelated to the study drug) was similar among the six treatment groups; the highest event rate with bosentan was 43 percent (in the 2000-mg group), as compared with 37 percent with placebo and 34 percent with enalapril. The most common adverse events reported with bosentan were headache (highest event rate, 24 percent with the 2000-mg dose, as compared with 18 percent with placebo), flushing (highest event rate, 18 percent with the 2000-mg dose, as compared with 4 percent with placebo), and leg edema (highest event rate, 14 percent with the 2000-mg dose, as compared with 0 percent with placebo). The rate of adverse events on day 1 (mainly headache and flushing) was higher with bosentan (20 percent with the 2000-mg dose) than with placebo (4 percent). No serious adverse events were reported.

A small number of patients receiving bosentan had asymptomatic increases in serum alanine and aspartate aminotransferase levels (one patient receiving 100 mg, one receiving 500 mg, two receiving 1000 mg, and four receiving 2000 mg). Six other patients (five receiving bosentan and one receiving placebo) had asymptomatic elevations of one aminotransferase or the other. An abnormal serum aminotransferase value was defined as an increase from base line of more than 50 percent and an absolute value of more than two times the upper limit of the normal range. These liver-function abnormalities were not associated with clinical sequelae and were fully reversible on cessation of the study treatment.

Discussion

Our study demonstrates that the administration of a specific endothelin-receptor antagonist, bosentan, in patients with mild-to-moderate essential hypertension results in a significant reduction in blood pressure as compared with placebo. These findings suggest that endothelin-1 contributes to elevated blood pressure in such patients. The reduction in blood pressure observed with a daily dose of 500 mg or more of bosentan was similar to that observed with a daily dose of 20 mg of enalapril.

Assessment of four doses of bosentan, with the highest dose 20 times the lowest dose, showed that a plateau in blood-pressure reduction was reached at a daily dose of 500 mg, suggesting that the reductions observed (diastolic pressure, 3.9 to 5.7 mm Hg; systolic pressure, 7.4 to 10.3 mm Hg) were near the top of the dose-response curve for bosentan.

The results of ambulatory blood-pressure monitoring suggest that the full antihypertensive effects of doses of bosentan administered once daily did not persist for the entire 24-hour period. Since bosentan has an elimination half-life of 4 to 10 hours, [16] these findings suggest that hemodynamic effects do not occur after the period of endothelin-receptor occupancy by the receptor antagonist.

It has been suggested that the relatively flat dose-response curve with bosentan is due to progressive blockade of the vasodilatory endothelial-cell ET_B receptor. Although this is theoretically possible, and bosentan is considered a mixed endothelin-receptor antagonist, the concentration that inhibits 50 percent binding of radiolabeled endothelin is much lower for the ET_A receptor than for the ET_B receptor, making a major contribution of endothelial ET_B-receptor blockade unlikely. A more likely explanation is a pharmacokinetic property of bosentan itself. A plateau in plasma drug levels has been reported with long-term therapy, and doses higher than 500 mg do not result in plasma levels that are significantly higher than those with lower doses. [16]

The mechanism by which treatment with an endothelin-receptor antagonist causes a reduction in blood pressure is uncertain, but a possible mechanism is direct peripheral vasodilatation due to blockade of the vasoconstrictor effects [1,17] of endothelin-1 on peripheral vascular smooth-muscle cells, reduced cardiac output, or both. Reduced cardiac output is unlikely, since recent data support an increase in cardiac output with long-term bosentan therapy, at least in patients with chronic heart failure. [18]

The blood-pressure reductions associated with endothelin-receptor antagonism in our study were not accompanied by reflexive increases in the heart rate. Drugs that act primarily as direct peripheral vasodilators (such as dihydropyridine calcium antagonists) are generally associated with activation of reflexive neurohormonal mechanisms, leading to increases in the heart rate as a homeostatic maneuver to restore blood pressure. [19] Activation of the renin-angiotensin and sympathetic nervous systems may signify an adverse prognosis and may account (at least in part) for the increased morbidity and mortality observed in epidemiologic studies of short-acting dihydropyridines. [20]

The absence of an increase in the heart rate with bosentan suggests the absence of reflexive neurohormonal activation with endothelin-receptor antagonism, and this suggestion was supported by the neurohormonal data. No significant increases in plasma levels of norepinephrine (reflecting the status of the sympathetic nervous system) or in plasma renin activity or plasma levels of angiotensin II (reflecting the status of the renin-angiotensin system) were observed with bosentan therapy. Thus, endothelin-receptor antagonism, despite lowering systemic blood pressure, did not appear to be associated with reflexive activation of either the sympathetic nervous system or the renin-angiotensin system. This finding has important implications for the therapeutic value of endothelin-receptor antagonists in a variety of cardiovascular diseases, particularly chronic heart failure, a condition associated with marked neurohormonal vasoconstrictor activation. [21,22]

The mechanisms underlying the inhibitory effect of endothelin-receptor antagonism on sympathetic and renin-angiotensin responses to reductions in blood pressure have not been clearly elucidated. Endothelin has facilitative effects on both systems [23,24]; blockade of the endothelin pathway through endothelin-receptor antagonism may inhibit these effects.

Blockade of ET_A) and ET_B) receptors with bosentan resulted in significant increases in plasma endothelin-1 levels, by approximately 50 percent. Angiotensin-converting-enzyme inhibitors have been associated with much larger reactive increases in plasma renin activity. In our study, plasma renin activity increased by almost 300 percent. Increases in plasma levels of endothelin-1 occurred even with the lowest dose of bosentan administered (100 mg daily), suggesting that a degree of receptor antagonism is maintained with long-term therapy at this low dose, although it is not sufficient to cause significant reductions in office measurements of systolic or diastolic pressure at trough drug levels.

In summary, our study demonstrates that long-term treatment with the endothelin-receptor antagonist bosentan in patients with mild-to-moderate hypertension results in significant reductions in blood pressure as compared with placebo. These findings suggest that endothelin-1 contributes to blood-pressure elevations in such patients. However, the magnitude of the contribution of endothelin-1 to blood pressure in patients with essential hypertension, as compared with persons with normal blood pressure, cannot be determined from this study.

Supported by a grant from Hoffmann-LaRoche.

Appendix

In addition to the authors, the following investigators participated in the study: K. Andreasen, Arhus, Denmark; P.J.L.M. Bernink, Groningen, the Netherlands; S. Braun, Tel Aviv, Israel; M. Brown, Cambridgeshire, United Kingdom; M. Bursztyn, Jerusalem, Israel; S. Carney, Newcastle, Australia; I.B. Fraemohs, Allingabro, Denmark; C. Hoeglund, Stockholm, Sweden; I. Kantola, Turku, Finland; Y. Karpov, Moscow, Russia; A. Kristinsson, Reykjavik, Iceland; A. Laszt, Ashqelon, Israel; J. Lenis, Longueil, Que., Canada; A. Schelling, Rotterdam, the Netherlands; and J. Stephens, Romford, United Kingdom.