Propranolol Increases Prostacyclin Synthesis in Patients with Essential Hypertension
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SUMMARY We tested the hypothesis that vascular prostacyclin synthesis is increased by propranolol and could account for some of the drug's antihypertensive effect. We studied 10 white patients with mild essential hypertension in a randomized, double-blind design to assess the effects of indomethacin with or without the addition of propranolol on blood pressure and vascular prostacyclin biosynthesis, as assessed by the urinary excretion of the major enzymatically produced metabolite of prostacyclin, 2,3-dinor-6-keto-prostaglandin F₁α (PGF₁α), measured by gas chromatography–mass spectrometry. Seven patients responded to propranolol with a lowering of mean arterial blood pressure in both supine and upright postures. The fall in mean arterial blood pressure (–14.1 ± 2.1 mm Hg sitting; –17.4 ± 1.7 mm Hg supine) with propranolol alone was significantly greater than that produced when propranolol was given to patients receiving indomethacin (–7.8 ± 1.9 mm Hg sitting; –7.7 ± 3.0 mm Hg supine). Our drug-responsive patients demonstrated a significantly lower excretion rate of 2,3-dinor-6-keto-PGF₁α than was found in an age and sex-matched group of normal volunteers. With propranolol treatment, drug-responsive patients showed a significant increase in the excretion of 2,3-dinor-6-keto-PGF₁α, such that the mean excretion was not significantly different from that in normal volunteers. Indomethacin caused a significant rise in mean arterial blood pressure and a significant fall in 2,3-dinor-6-keto-PGF₁α excretion, and it blocked the rise in urinary 2,3-dinor-6-keto-PGF₁α associated with propranolol therapy. The patients with an antihypertensive response to propranolol had a significant negative correlation between their baseline mean arterial blood pressure and 2,3-dinor-6-keto-PGF₁α excretion. The three nonresponders to propranolol did not display such a relationship between mean arterial blood pressure and 2,3-dinor-6-keto-PGF₁α, and they had only a small increase in 2,3-dinor-6-keto-PGF₁α excretion with propranolol, such that 2,3-dinor-6-keto-PGF₁α levels remained significantly less than mean normal values, even in the face of propranolol therapy. These findings suggest that enhanced synthesis of prostacyclin is associated with the full antihypertensive effect of propranolol. In addition, the hypertensive effect of indomethacin, by whatever mechanism, blunts the full antihypertensive response to propranolol. (Hypertension 12: 582–588, 1988)

KEY WORDS • prostacyclin • indomethacin • 2,3-dinor-6-keto-prostaglandin F₁α • antihypertensive agents • mass spectrometry

NUMEROUS studies demonstrate that nonsteroidal antiinflammatory drugs (NSAIDs) interfere with the antihypertensive efficacy of several therapeutic agents, but the exact role of prostaglandins in modulating the efficacy of certain antihypertensive drugs is not well defined. In 1975, Patak et al. reported antagonism of the antihypertensive effect of furosemide by indomethacin. Subsequently, several investigators have reported that NSAIDs attenuate the blood pressure–lowering effect of thiazide diuretics, β-adrenergic blockers, vasodilators, and converting enzyme inhibitors. Some, but not all, investigators have reported that NSAIDs increase blood pressure in normotensive and untreated essential hypertensive subjects. The mechanism of any hypertensive effect of such prostaglandin cyclooxygenase inhibitors is unclear, and attempts to correlate the effect with suppression of systemic or renal prostaglandin production have been inconclusive. Considerable indirect evidence indicates that vasodilator prostaglandins produced within blood vessels modulate the effect of vasoconstrictors to increase vascular
resistance. The loss of this "buffer system" through cyclooxygenase inhibition may result in the attenuation of the antihypertensive effect of several drugs.

When the β-adrenergic blocking drug propranolol is first administered, there is a fall in cardiac output and a rise in peripheral vascular resistance, with little change in arterial pressure. With continued propranolol administration, the arterial pressure falls coincidently with a return of vascular resistance to the baseline level. The mechanism of this delayed fall of vascular resistance and arterial pressure is unknown. The current study was designed to explore the hypothesis that vascular prostacyclin (PGI₂) synthesis is increased by propranolol administration and accounts for some of the drug's antihypertensive effect.

We therefore asked a number of questions in this study: Does the β-blocker propranolol enhance the synthesis of the vasodilator prostaglandin PGI₂, as measured by the excretion of the major urinary metabolite of PGI₁ (PGIM), 2,3 dinor-6-keto-prostaglandin F₉ₓ (PGF₉ₓ)? Is PGI₂ synthesis required for the full antihypertensive efficacy of propranolol? Is the interaction between indomethacin and propranolol due to a hypertensive action of the NSAID or a diminished antihypertensive action of the β-blocker (or both)? Is this interaction between NSAIDs and β-blockers due to salt retention, as reflected by an increase in body weight?

Patients and Methods

Patient Population

Ten white patients (aged 39 to 63 years; five men and five nonpregnant women) with mild chronic essential hypertension were studied in a randomized, double-blind, crossover trial that was approved by the Human Subjects Committee, University of Colorado Health Sciences Center. Hypertension was defined as a diastolic blood pressure of 95 to 105 mm Hg and a systolic blood pressure of 135 to 165 mm Hg. The patients were screened as outpatients in the Clinical Research Center on the basis of a history, physical examination, serum electrolyte, creatinine, and glucose levels, electrocardiogram, urinalysis, and a pregnancy test for the female patients. All results were normal. No patient had any evidence or history of acute or chronic heart, lung, liver, or renal diseases, peptic ulceration, aspirin allergy, or diabetes. Patients agreed to refrain from taking any medication throughout the study. Each patient signed an informed consent before study enrollment.

Consenting patients were randomized into two groups. Each group was removed from all medications for at least 2 weeks, following which each group received either placebo or indomethacin, 50 mg b.i.d., in a double-blind fashion. After 1 week of either placebo or indomethacin, propranolol, 100 mg b.i.d., was administered to all patients in addition to the regimen of placebo or indomethacin. After 2 weeks of placebo or indomethacin plus propranolol, all drugs were discontinued for a 2-week washout period. After this second washout, all patients crossed over and repeated the study with the opposite regimen of placebo or indomethacin followed by the addition of propranolol. All patients were maintained on a 150 to 200 mEq/day salt diet throughout the study. Patients' urinary sodium excretion averaged 157 ± 16.5 mEq/24 hr (range, 116-283 mEq/24 hr).

Each patient was seen weekly during washout and indomethacin or placebo periods and twice weekly during antihypertensive therapy. At each clinic visit, weight and arterial blood pressure were measured; blood was assayed for electrolytes, blood urea nitrogen, and creatinine; and a 24-hour urine was collected for PGIM, creatinine, and sodium excretion. Propranolol levels were measured as an index of patient compliance. Blood pressure was taken in both supine and upright positions with a mercury manometer cuff by the same investigators in the Clinical Research Center.

PGI₁ Metabolite Measurements

PGIM was measured by stable isotope dilution using a gas chromatography–negative ion, chemical-ionization mass spectrometry technique, as developed by Brash et al.14 and modified by FitzGerald et al.15, 16 The urinary excretion of PGIM reflects total body PGI₂ synthesis. Briefly, deuterated internal standard PGIM, 1 ng/ml, was added to duplicate urine aliquots from each patient. The urine was stored at -70°C until extraction and assay. A 20-ml sample was extracted and back-extracted with a series of solvent solutions under alkaline and acidic conditions.15 The final extracted PGIM was then derivatized by methoximation and formation of the pentfluorobenzyl ester.13 The derivative was purified by thin-layer chromatography, and then the trimethylsilyl ether derivative was formed prior to final chromatographic–spectrometric analysis.16 Quantitative measurements were performed using a Nermag 10-10C mass spectrometer (Delsi, Ruei-Malmaison, France) interfaced with a Varian 3400 gas chromatograph (Palo Alto, CA, USA) fitted with a DB-1 methylsilicone capillary chromatography column (J&W Scientific, Folsom, CA, USA). Quantitation was accomplished by monitoring m/z 586 for endogenous PGIM and m/z 590 for the deuterium-labeled internal standard. Assay precision was 95.6 ± 3.0%. The interassay variability was 11.0 ± 2.2%, and the intra-assay variability was 5.0 ± 0.9%.

Statistical Analysis

The data were expressed as means ± SEM. Mean arterial blood pressure (MABP), sitting and supine, and PGIM excretion from placebo and indomethacin treatment periods were compared by a two-way analysis of variance. Dunn's test for multiple comparisons was used to compare the effects of propranolol and indomethacin treatments with control values. Duncan's test was applied to compare
treatment groups’ mean difference within the indomethacin and placebo periods. Finally, Student’s t test was applied to placebo and indomethacin periods to assess the change in PGIM excretion and weight changes in response to propranolol treatment under the two conditions of the study. Statistical difference was accepted if the p value was less than 0.05. MABP from all placebo and control measurements were compared with that observed during PGIM excretion using regression analysis by Statistical Analysis System-directed analysis of variance.

Results

Propranolol was an effective antihypertensive agent in 7 of our 10 study patients. Three of the 10 patients were termed nonresponders to propranolol due to a failure of propranolol alone to lower the MABP by at least 5 mm Hg in both supine and sitting positions. This determination was made before the randomization code was broken and the PGIM values were obtained. Data from the seven drug responders were analyzed separately from those of the three nonresponders.

In the drug responders, propranolol lowered the MABP in both the sitting and supine positions, as shown in Figure 1. In these same patients, indomethacin alone resulted in a significant rise in MABP in the upright and supine positions (p < 0.05). The subsequent addition of propranolol to indomethacin resulted in a significant fall in MABP in both positions (p < 0.01 upright, p < 0.05 supine). However, the fall in MABP produced by the addition of propranolol to placebo (−14.1 ± 2.1 mm Hg sitting, −17.4 ± 1.7 mm Hg supine) was significantly greater (p < 0.01) than that produced when propranolol was added to indomethacin (−7.8 ± 1.9 mm Hg sitting, −7.7 ± 3.0 mm Hg supine). Therefore, in addition to its hypertensive effect, indomethacin reduced the antihypertensive effect of propranolol. Because indomethacin increased MABP and reduced the antihypertensive effect of propranolol, the combination of indomethacin and propranolol resulted in no overall reduction in MABP, as compared with control values and a significantly (p < 0.01) higher MABP when compared with the effect of propranolol alone. There was no difference between the control and placebo MABP in either position or between the control values during the two parts of the study. There was no significant difference between the plasma propranolol concentrations in the absence (176 ± 54 ng/ml) or presence (152 ± 35 ng/ml) of indomethacin.

Although a slight weight gain was associated with indomethacin treatment, it was not significant (−0.63 ± 0.49 kg with placebo vs +0.86 ± 0.32 kg with indomethacin). There was a significant weight gain when propranolol was added to indomethacin as compared to the gain produced by propranolol with placebo (+1.57 ± 0.59 kg vs +0.26 ± 0.10 kg, p < 0.01).

The results of the PGIM analysis are shown in Figure 2. Our untreated hypertensive patients had a mean PGIM excretion that was significantly less than that from a similarly sized group of age and sex–matched normal volunteers (59.9 ± 21.7 vs 185.0 ± 33.5 ng/g, p < 0.05). Propranolol treatment alone was associated with a highly significant increase in PGIM excretion (p < 0.01), such that...
our treated responder patients attained PGIM levels that were equal to those of the untreated normal volunteers (135.6 ± 17.6 vs 185.0 ± 33.5 ng/g). Indomethacin administration resulted in a reduced excretion of PGIM (p < 0.01). Indomethacin also blocked the rise in PGIM excretion associated with propranolol treatment, and the difference between placebo and indomethacin and propranolol and indomethacin treatment groups was highly significant (p < 0.01) in the responder group.

The three patients who did not have a clinical hypotensive response to propranolol showed control and placebo PGIM values similar to those of the responders (control: 54.5 ± 8.9 ng/g for nonresponders vs 59.9 ± 21.7 ng/g for responders, placebo: 40.2 ± 1.6 ng/g for nonresponders vs 63.2 ± 12.7 for responders). However, the nonresponders showed only a small rise in PGIM during propranolol therapy (+28.7 ± 10.7 ng/g) that was significantly less (p < 0.05) than that achieved by the responders (+71.2 ± 13.3 ng/g).

Figure 3 shows the relationship between PGIM excretion and sitting MABP in the responders in the absence of any treatments. Interestingly, there was a significant correlation (p < 0.05) between PGIM and MABP in all nonmedicated patients who subsequently had an antihypertensive response to propranolol. However, the three nonresponders did not show this same relationship between MABP and PGIM excretion during any of the study periods (data not shown).

Discussion

In this study, propranolol therapy was associated with a marked enhancement of extrarenal PGI₂ production, as assessed by the urinary excretion of PGIM. Brash et al. and others have demonstrated that PGIM is the major enzymatic metabolite excreted in the urine in humans and that the excretion rate of this metabolite reflects the extra-renal synthesis of PGI₂. Further, since the major source of PGI₂ is thought to be the vascular endothelium, an increase in the excretion of the PGIM implies an increase in vascular PGI₂ synthesis. Because of the difficulty in measuring PGIM, most investigators have measured urinary 6-keto-PGF₁α to assess PGI₂ status. However, in contrast to PGIM, 6-keto-PGF₁α is a nonenzymatic hydrolysis product of PGI₂ that may arise in the kidney or elsewhere and bears no obvious or consistent relation to vascular PGI₂ synthesis. One potential pitfall in our use of PGIM as an index of PGI₂ production is the possibility that propranolol may have changed the metabolic profile of PGI₂, such that an increase in urinary excretion of PGIM occurred in the absence of any real synthesis enhancement. To our knowledge, there are no reports of such a metabolic shunting of PGI₂, and we doubt that such a hypothesis explains our finding of a more than twofold increase in PGIM excretion with propranolol therapy.

When the cyclooxygenase inhibitor indomethacin was present, propranolol therapy did not increase
PGI₂ production or decrease arterial pressure below control values. This inhibition of propranolol's anti-hypertensive effect by indomethacin was due to two interacting factors. First, indomethacin alone increased the arterial pressure in these patients; second, indomethacin attenuated the reduction of arterial pressure produced by propranolol. This finding suggests that propranolol lowered blood pressure by both prostaglandin-dependent and prostaglandin-independent mechanisms. The return of the elevated MABP to control values by propranolol administration in indomethacin-treated subjects was not contingent on prostaglandin synthesis, since it was not associated with an increased excretion of PGIM. However, the additional antihypertensive effect of propranolol that was seen in the absence of indomethacin was associated with an increase in PGI₂ production and thus may have been at least partially dependent on an increased synthesis of vasodilator prostaglandins.

Although we found an increase in the PGIM excretion with propranolol administration in the seven patients who showed an antihypertensive response to propranolol, PGIM also rose slightly in the three patients who did not respond to propranolol therapy. Whereas propranolol therapy elevated PGIM levels in the drug responders to the equivalent of levels in the untreated normal volunteers, the small rise in PGIM in the nonresponders was not associated with normalization of either blood pressure or PGIM excretion. This observation suggests that the elevation of vascular PGI₂ production to normal levels may contribute to a hypotensive response to propranolol. Alternatively, the relative hypotension induced by propranolol may have caused the increased PGI₂ synthesis in responding patients.

In addition to contributing to the hypotensive action of propranolol, increased vascular PGI₂ production could potentially explain the reported ability of propranolol to normalize platelet aggregability in patients with angina pectoris who had hyperaggregable platelets. Although high concentrations of propranolol can inhibit platelet aggregation in vitro by a direct effect on membranes, such an explanation is unlikely for propranolol concentrations achieved in vivo, which are not high enough for membrane effects.

The ability of indomethacin to increase blood pressure in propranolol-treated patients has been reported previously. None of the early studies considered the possibility that indomethacin might have a direct hypertensive effect that subtracted from the effect of propranolol, since they did not examine the effect of indomethacin alone. Several recent studies, including ours, have reported a hypertensive effect of indomethacin in patients with essential hypertension, often associated with a small weight gain. Although, to our knowledge, no previous clinical studies have attempted to assess the ability of propranolol to stimulate vascular PGI₂ production, data accumulated from studies in vitro indicate that propranolol may have the ability to increase PGI₂ production in the vascular endothelium.

In addition to showing the ability of propranolol to increase PGI₂ production in humans, our study shows a significant negative correlation between the excretion of PGIM and basal blood pressure in nonmedicated patients who subsequently responded to propranolol. This association suggests that there may be some hypertensive patients with low basal PGI₂ synthesis in whom an increase in synthesis of vascular vasodilator prostaglandins by propranolol or other drugs may be accompanied by beneficial effects in blood pressure control. The only other clinical report of a relationship between PGI₂ production and arterial pressure is that of Fitzgerald et al., who reported that women who became hypertensive during pregnancy exhibited a lesser increment in PGI₂ production than did healthy pregnant subjects. These data have a limited relationship to our findings, since pregnancy-induced hypertension is quite different from essential hypertension. One potentially relevant animal model of human essential hypertension, the Dahl salt-sensitive rat, has been shown to have a defect in PGI₂ production compared with the salt-resistant strain, as assessed by the urinary excretion of the same PGIM that we measured in our study. The defect was demonstrated by feeding the animals a high salt diet, which resulted in an impaired PGI₂ synthesis that preceded the elevation in arterial pressure produced by the increased salt intake.

In our study, the interaction between indomethacin and propranolol appears to be due to a hypertensive effect of indomethacin and a blunting of the antihypertensive effect of propranolol. Sodium retention attributable to indomethacin may have contributed to these effects. There was a significant weight gain during indomethacin plus propranolol as compared with propranolol treatment alone. The mechanism of NSAID-induced sodium retention is unclear, but considerable evidence favors increased proximal tubular reabsorption of sodium and water. Sodium retention leads to expanded extracellular volume, which in the essential hypertensive patient, has been linked to elevated blood pressure and a reduced response to diuretics and adrenergic blockers. This phenomenon may have contributed to our patients' attenuated responses to propranolol in the presence of indomethacin.

In addition to their sodium-retaining properties, the NSAIDs may also cause elevated blood pressure by inhibition of vascular prostaglandin synthesis. In our study, when indomethacin was given alone, it was not associated with a significant weight gain, although there was a significant rise in blood pressure. Several studies have suggested an increased systemic vascular resistance and an increased sensi-
tivity to vasoconstrictors (i.e., angiotensin II and norepinephrine) as the underlying mechanisms for this hypertensive effect of NSAIDs. Another possibility is that indomethacin has direct vascular effects that increase vascular resistance and are unrelated to prostaglandin inhibition, as proposed by Edlund et al. in the coronary circulation.

In conclusion, we have demonstrated a propranolol-associated increase in total body vascular PG\textsubscript{12} synthesis in all patients with or without a hypertensive response to propranolol. However, the propranolol-associated increase in PG\textsubscript{12} synthesis was greater in those patients who had an antihypertensive response to propranolol than in those patients who remained hypertensive with propranolol therapy. There was an inverse relationship between basal systemic PG\textsubscript{2} activity to vasoconstrictors (i.e., angiotensin II and norepinephrine) as the underlying mechanisms for this hypertensive effect of NSAIDs. Another possibility is that indomethacin has direct vascular effects that increase vascular resistance and are unrelated to prostaglandin inhibition, as proposed by Edlund et al. in the coronary circulation.

Acknowledgments

The authors express their appreciation to the Clinical Mass Spectrometry/National Institutes of Health Resource for analytical support during this study and to Dr. Robert Murphy for critical review of this manuscript.

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Hypertension. 1988;12:582-588
doi: 10.1161/01.HYP.12.6.582

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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