Suppression of Plasma Renin Activity by Indomethacin in Man

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SUMMARY The effect of indomethacin or placebo on aldosterone, plasma renin activity (PRA), sodium excretion, and urinary prostaglandin (PG) levels was investigated in five hypertensive subjects in 100 mEq sodium balance who had experienced malignant hypertension with a disturbance of their renin-aldosterone relationship in the past. Indomethacin significantly lowered aldosterone levels by 43%, PRA by 58%, 24-hour sodium excretion by 49%, and urinary PG excretion, an indicator of renal PG synthesis, by 67%. Angiotensin infusion increased aldosterone to the same level before and after treatment with indomethacin. Similarly, in normal subjects in 150 mEq sodium balance, indomethacin lowered PRA by 47%; sodium excretion fell by 33%, and urinary prostaglandin E (PGE) excretion, by 55%. The acute elevation in PRA 10 minutes after intravenous furosemide was completely abolished by indomethacin. Five subjects with essential hypertension were classified as normal renin hypertensives according to their response to orally administered furosemide. Indomethacin pretreatment resulted in 60% reduction of PRA following furosemide, and three of these subjects now fell into the low renin category. Studies in vitro demonstrated that indomethacin has no effect on the renin-renin substrate interaction. Thus, indomethacin lowers PRA concomitantly with a reduction in renal PG synthetase activity. Whether indomethacin inhibits renin release by an intrarenal, PG-related mechanism or secondarily via sodium retention is discussed.

Methods

STUDIES IN MAN

Patients with Postmalignant Hypertension. Five subjects who previously had had malignant hypertension characterized by severe hypertension (diastolic blood pressure greater than 130 mm Hg), papilledema, and signs of microangiopathic hemolytic anemia were hospitalized. At this time one subject still exhibited increased urinary aldosterone secretion without increased PRA, whereas the other subjects had normal aldosterone secretion rates. Further data on these subjects are given in Table 1. Informed consent for the study that had been approved by the Vanderbilt Clinical Investigation Committee was obtained. The subjects were brought into 100 mEq sodium balance and a placebo capsule resembling 25 mg of indomethacin was administered four times daily for 5 days. On the 4th day, a 24-hour urine was collected for determination of sodium, creatinine, and PGE. On the 5th day, after a 4-hour control period during which the vehicle (5% glucose in water) was given intravenously, angiotensin II amide (Hypertensin, Ciba) was infused at an average rate of 1.52 ng/kg per min for 4 hours. In the middle of the control and angiotensin infusion period, blood was obtained for the determination of PRA, aldosterone, and creatinine. Urine was collected during each period. Blood pressure was monitored every 15 minutes throughout the study. Subsequently, each subject received the active drug (25 mg four times a day) for 4 days and an infusion of the same dose of angiotensin II amide was repeated on the 5th day.

Normal Volunteers. Five male volunteers were screened by history, physical examination, sequential multiple anal-
STUDIES IN VITRO

Studies in vitro were performed to determine whether indomethacin or its metabolites would interfere with PRA determination. To duplicate samples of pooled human plasma, indomethacin was added in increasing concentrations (0, 10, 100, and 10,000 ng/ml) and the PRA was assayed. To investigate whether any metabolites of indomethacin found in plasma might interfere with PRA measurement, a patient with primary aldosteronism was treated with 50 mg of indomethacin four times daily for 2 days. Two hours after the last dose, blood was collected and to the plasma of this blood were added 2 x 10^{-4} Goldblatt units of human kidney renin. The generation of angiotensin I from this plasma was compared to angiotensin I generation in the plasma of this subject obtained prior to indomethacin treatment to which an identical amount of human renin had been added.

PRA was determined as described previously. Aldosterone was determined by radioimmunoassay and urinary PGE by competitive protein-binding assay. Furosemide was determined by high performance liquid chromatography (unpublished observations). Statistical analysis was accomplished by Student’s t-test.

Results

EFFECT OF INDOMETHACIN ON PLASMA ALDOSTERONE AND PRA IN POSTMALIGNANT HYPERTENSION

Indomethacin reduced plasma aldosterone levels measured during the control period by 43% from 11.4 ± 6.8 (mean ± SD) to 6.5 ± 2.9 ng/100 ml (P < 0.05) (Fig. 1). The simultaneously determined PRA fell in each of four subjects from 5.7 ± 2. to 2.4 ± 0.89 ng of angiotensin I/ml per hour (P < 0.05, Wilcoxon test) (Fig. 1). In the fifth subject the effect of indomethacin on PRA could not be evaluated because he had suppressed PRA (PRA < 0.17 ng of angiotensin I/ml per hour) throughout the study. Indomethacin reduced urinary sodium excretion from 110.9 ± 20.2 (average of the 2 days preceding indomethain) to 56.8 ± 18.9 (average of the first 2 days on indomethacin) mEq/24 hours (P < 0.05) (Fig. 1); this was accompanied by an average weight gain of 0.8 ± 0.1 kg (P < 0.02). Urinary

TABLE I Clinical Data for Subjects with Postmalignant Hypertension

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Treatment</th>
<th>Blood pressure (mm Hg)</th>
<th>Years since last episode*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>62</td>
<td>GE (25 mg)</td>
<td>180/100</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>31</td>
<td>AMD (250 mg)</td>
<td>130/100</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>58</td>
<td>GE (25 mg)</td>
<td>180/100</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>53</td>
<td>AMD (500 mg)</td>
<td>160/85</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>42</td>
<td>GE (20 mg)</td>
<td>196/108</td>
<td>2</td>
</tr>
</tbody>
</table>

GE = guanethidine; AMD = α-methyldopa
* Years elapsed since last episode of malignant hypertension
† Diuretic (one capsule) consisted of 25 mg of hydrochlorothiazide and 50 mg of triamterene.

analysis of 18 parameters (SMA-18), urinalysis, chest x-ray, and electrocardiogram and found to have no abnormalities. They were brought into 150 mEq sodium balance and received in randomized, crossover fashion either placebo or 50 mg of indomethacin three times daily for 2 days and one capsule at 7 a.m. on the 3rd day. On the 2nd day a 24-hour urine was collected for sodium, creatinine, and PGE determination. On the 3rd day the volunteers were kept ambulatory from 6 a.m. to 9 a.m., and at 9 a.m. blood was collected for PRA determination. Five minutes later 20 mg of furosemide was given as a bolus intravenously, and blood was collected for PRA determination. Five minutes after drug administration. The level of furosemide was determined in the plasma collected 10 and 30 minutes after its administration. In the urine excreted in the 2 hours following furosemide administration, sodium, creatinine, PGE, and furosemide were measured.

Hypertensive Subjects with Normal PRA. Five subjects with essential hypertension and normal PRA (who also had normal renal arteriograms, normal urinary excretion of 17-keto steroids and 17-hydroxy steroids, creatinine clearance, vanillylmandelic acid, and catecholamines) were selected for evaluation of the effect of indomethacin on the PRA response to furosemide administered orally according to the procedure used in the categorization of hypertensive subjects with low renin hypertension. According to this test these subjects were found to have normal PRA. They also had normal PRA according to the criteria established by Brunner et al., which are based on the sodium-renin index. In the former test, the subject receives 40 mg of furosemide orally 15, 9, and 3 hours prior to PRA sampling and is kept upright after administration of the third dose. A PRA value >1.67 ng of angiotensin I/ml per hour is considered indicative of normal renin hypertension. The responsiveness of each subject’s PRA to this test had been established on at least two occasions prior to our present study and on each occasion the PRA was >1.67 ng angiotensin I/ml per hour. The PRA response to the same dose of furosemide in these subjects was tested when they were on no drugs, after they had received 50 mg of indomethacin every 6 hours starting 45 hours prior to PRA sampling, and 1 week later while receiving no drugs.
EFFECT OF INDOMETHACIN ON PRA BEFORE AND AFTER INTRAVENOUSLY ADMINISTERED FUROSEMIDE IN NORMAL VOLUNTEER SUBJECTS

In the normal volunteer subjects in balance on a 150 mEq sodium diet indomethacin caused a fall in control PRA from $5.3 \pm 1.4$ to $2.8 \pm 1.6$ ng of angiotensin I/ml per hour ($P < 0.05$) (Fig. 2), and sodium excretion fell from $174 \pm 15.6$ to $115.9 \pm 51.5$ mEq/24 hours ($0.1 > P > 0.05$) in parallel with urinary PGE excretion from $80.1$ to $36.4$ ng/hour ($P < 0.05$, Wilcoxon test).

Intravenous furosemide caused a steep rise in PRA that reached maximum 10 minutes after administration of the drug and was completely abolished by indomethacin (Fig. 2). Sodium excretion during the 2 hours after administration of furosemide was $122 \pm 8$ mEq for the placebo period and $88 \pm 12.6$ mEq for the indomethacin period ($P < 0.01$).

Furosemide plasma levels during the placebo period, 10 and 30 minutes after its administration, were $4.56 \pm 0.59$ 
 microg/ml and $1.38 \pm 0.32$ 
 microg/ml, respectively. During the indomethacin period the levels were $5.3 \pm 1.3$ and $1.64 \pm 0.3$ 
 microg/ml, respectively ($P$ not significant). Furosemide concentration in the urine excreted during the 2 hours following administration of the drug was $11.44 \pm 2.46$ 
 microg/ml during the placebo period and $12.2 \pm 1.98$ 
 microg/ml during the indomethacin period. The amount of furosemide excreted in the urine during this period was $11.9 \pm 1.7$ mg during the placebo period and $9.8 \pm 1.3$ mg during the indomethacin period ($P < 0.01$).

EFFECT OF INDOMETHACIN ON THE TEST USED TO CATEGORIZE LOW RENIN HYPERTENSION (PRA RESPONSE TO ORALLY ADMINISTERED FUROSEMIDE)

The effects of indomethacin on the furosemide-induced PRA increase and weight loss are shown in Table 3. The PRA increase after furosemide was decreased by indomethacin by 60% ($P < 0.01$). During the indomethacin treatment period, three out of five subjects failed to increase their PRA to a level greater than 1.67 ng of angiotensin I/ml per hour and thus appeared to fall into the category of low renin hypertensives. Indomethacin reduced furosemide-induced weight loss by 63% ($P < 0.01$). Both responses returned to control levels 1 week after indomethacin was discontinued.

STUDIES TO EXCLUDE AN EFFECT OF INDOMETHACIN ON THE IN VITRO GENERATION OF ANGIOTENSIN I BY RENIN

The effect of three concentrations of indomethacin on the synthesis of angiotensin I in plasma is shown in Table 4. Indomethacin did not inhibit PRA in concentrations ranging from 10 to 10,000 ng/ml of plasma.

The synthesis of angiotensin I in the plasma of a subject with primary aldosteronism to which exogenous human renal renin had been added was $3.46 \pm 0.55$ ng of angiotensin I/ml per hour before and $3.88 \pm 0.41$ ng during treatment with indomethacin ($n = 6$, $P$ not significant).
TABLE 2  Effect of Indomethacin on Aldosterone (ng/100 ml), Plasma Renin Activity (PRA) (ng Angiotensin I/ml per hour), and Prostaglandin E (PGE) Excretion Rate (ng/hour) Before (Control) and During Angiotensin Infusion in Five Subjects with Postmalignant Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>Angiotensin (n = 4)</th>
<th>P (control/angiotensin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Aldosterone</td>
<td>PRA</td>
<td>PGE</td>
</tr>
<tr>
<td></td>
<td>11.4 ± 6.8</td>
<td>171 ± 30.2</td>
<td>25.7 ± 16.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>6.5 ± 6.6</td>
<td>69.8 ± 24.6</td>
<td>8.4 ± 5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo/indomethacin</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
NS = not significant.

Discussion

Indomethacin was found to lower PRA in subjects with hypertension, both essential and postmalignant, and in normal volunteer subjects. This effect was associated with a substantial reduction in the urinary excretion of PGE, a finding that probably mirrors a reduction in the synthesis of PGE in the kidney. In all instances the changes in PRA were associated with some degree of retention of sodium; this finding made it difficult to ascertain whether indomethacin lowered PRA by a mechanism confined to the kidney, by an increase in plasma volume, or both.

Evidence favoring a direct effect of PG's on renin release stems from our earlier observation that PGE, stimulates the release of renin from cell suspensions of rabbit renal cortex. Also, an acute inhibitory effect of indomethacin on renin release has been observed in the rabbit; the rapid reduction in PRA following the parenteral administration of indomethacin is not consistent with an exclusive role of sodium retention in the reduction of PRA. The time course of the PRA response to furosemide in the present study is of interest in that the highest (2.6-fold) rise in PRA was observed 10 minutes after drug administration. Thirty minutes after furosemide, at a time when much more sodium had been excreted, PRA was only 1.3-fold higher than during the control period. This suggests that part of the rise in PRA following the intravenous administration of furosemide is caused by factors other than reduction in the blood volume. This acute response is most significantly affected by blockade of PG synthesis and, thus, PG synthetase may be of importance in bringing about the rise in PRA following furosemide. Recently it has been shown that indomethacin blocks the acute increase in renal blood flow in the dog that follows bumetanide and furosemide administration. This observation suggests that furosemide can also alter intrarenal hemodynamics by a PG-mediated mechanism which is independent of blood volume. Current evidence is insufficient to determine whether indomethacin affected the acute release of renin by furosemide via a direct influence on the renin-releasing cell or indirectly via a change in renal hemodynamics or tubular-macula densa transport of electrolytes. Whatever the mechanism, indomethacin clearly produces significant changes in the body's volume control mechanism, and this is correlated with suppression of PG synthetase activity.

The possibility that indomethacin blocked the tubular secretion of furosemide and prevented its intraluminal action was considered. Probenecid, by inhibiting the secretion of furosemide, will antagonize its diuretic effect. If such an inhibition of furosemide's transport into the tubule were responsible for the total inhibition of early PRA release, then the renal elimination of furosemide should have been similarly prevented. However, in the present study the slight decrease in the rate of elimination of furosemide by indomethacin cannot account for the complete blockade of the PRA response.

The effect of indomethacin on PRA prompted us to investigate whether the reaction between renin and its
TABLE 4

<table>
<thead>
<tr>
<th>Indomethacin (ng/ml plasma)</th>
<th>PRA (ng A 1/1/ml per hr)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.42 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.22 ± 0.49</td>
<td>NS</td>
</tr>
<tr>
<td>100</td>
<td>5.92 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>10,000</td>
<td>6.07 ± 0.62</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD; A 1 = angiotensin I.
Red Cell Velocity and Plasma Transit Time in the Cerebral Microcirculation of Spherocytic Deer Mice

WILLIAM I. ROSENBLUM, M.D.

SUMMARY  Spherocytic deer mice provide a model of human spherocytosis. Their erythrocytes are abnormal in shape and are more rigid than normal red blood cells (RBC). Like their human counterparts, spherocytic mice are anemic. Measurements of RBC velocity in microvessels on the cerebral surface failed to reveal a difference between the velocity of cells in spherocytic as compared to normal deer mice. However, plasma transit, as measured by fluorescein microangiography, was faster than normal. Both decreased plasma transit time and increased RBC velocity are expected in nonspherocytic, anemic mice. Since the former, but not the latter, was found in the spherocytic, anemic mice, it appears that increased RBC rigidity has a greater effect on RBC movement than on plasma movement within the cerebral microcirculation. Thus it would seem that this increased RBC rigidity prevents the increased RBC velocity that otherwise would be observed in anemia but does not prevent the decrease in plasma transit time.

APPROXIMATELY 20 years ago hereditary spherocytosis was recognized in the deer mouse. The hematological, pathological, and physiological features of this syndrome were shown to be similar to those of spherocytosis in man. In spite of the interest one might expect in this model of a disease with abnormal red blood cells (RBC), little use appears to have been made of the affected animals for studies of blood rheology or of the microcirculation.

Our interest in the animal model of spherocytosis was prompted by our previous studies of the cerebral microcirculation in other models of rheological disorders. In these earlier investigations on mice we demonstrated that two hypertensive conditions, macroglobulinemia and polyethylenoma, exerted a greater effect on plasma flow than on RBC velocity. Thus plasma flow was retarded more than RBC velocity, a phenomenon that appeared to accentuate the normal difference between the velocity of cells in spherocytic and normal deer mice. However, plasma transit, as measured by fluorescein microangiography, was faster than normal. Both decreased plasma transit time and increased RBC velocity are expected in nonspherocytic, anemic mice. Since the former, but not the latter, was found in the spherocytic, anemic mice, it appears that increased RBC rigidity has a greater effect on RBC movement than on plasma movement within the cerebral microcirculation. Thus it would seem that this increased RBC rigidity prevents the increased RBC velocity that otherwise would be observed in anemia but does not prevent the decrease in plasma transit time.

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