Effect of Colchicine on Drug-Induced Changes in Plasma Renin Concentration in Rats

VICKI STYGLES DONOSO, M.S., AND MICHAEL D. BAILIE, M.D., PH.D.

SUMMARY Cytoplasmic microtubules appear to play a role in the secretion of a variety of protein and protein hormones. Involvement of microtubules in renin secretion has been hypothesized but not established. The present studies were designed to determine: 1) If the antimicrotubule drug, colchicine, would alter plasma renin concentration (PRC); and 2) if changes in PRC could be related to an effect on cytoplasmic microtubules. Dose response experiments in Sprague-Dawley rats showed that 0.4 or 0.8 mg/kg/day i.p. of colchicine for 3 days significantly increased PRC while a dose of 0.2 mg/kg/day was without effect. The increase in PRC at the higher doses was associated with toxicity of the drug. In other experiments, rats pretreated with colchicine (0.2 mg/kg/day) or saline received either furosemide (5 mg/kg) or isoproterenol (25 μg/rat) i.p. to stimulate renin secretion. Colchicine at a dose that did not alter basal PRC significantly inhibited an increase in PRC after stimulation with either isoproterenol or furosemide. Lumicolchicine, a structural isomer of colchicine without antimicrotubule activity, did not alter the response to isoproterenol stimulation. These data suggest that microtubules play a role in the increase in renin secretion following stimulation.

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KEY WORDS • renin-angiotensin system • lumicolchicine • cytoplasmic microtubules

THE intracellular system of cytoplasmic microtubules has been implicated in numerous cellular functions including the secretion of hormones and proteins. Because of the characteristic ability of the antimitotic drugs, colchicine and vinca alkaloids, to interact with microtubules, these drugs have been used to study the role of microtubules in secretory processes. In vivo and in vitro studies have demonstrated that antimitotic drugs inhibit secretion of insulin, retinol binding protein, parathyroid hormone, very low density lipoprotein, thyroxine, and milk from rat mammary gland. In many cases, inhibition of secretion was related to ultrastructural findings of microtubule disruption.

Several investigators have studied the effect of antimitotic drugs on renin secretion in an attempt to determine whether microtubules are involved with the release of this hormone from the kidney. The results of these experiments have been controversial, and no consistent effect of these drugs on the renin-angiotensin system has been established. Furthermore, these studies emphasized in vitro effects.

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Our present experiments were designed to determine the effect of treatment of rats with colchicine on plasma renin concentration (PRC). The studies were designed to minimize drug side effects that might alter renin secretion or metabolism and thereby modify renin concentration in plasma.

Methods

Colchicine is known to have many toxic effects which, in rats, include weight loss and diarrhea. Since these side effects could cause an alteration in PRC unrelated to effects on microtubules, a dose response experiment was performed to determine a dose of colchicine that minimized toxicity.

Twenty male Sprague-Dawley rats were randomly assigned to four treatment groups. Rats were individually housed in metabolism cages and had free access to food (0.4% Na+) and water. Each group of rats received either 0.9% saline or colchicine in 0.9% saline (0.2, 0.4, or 0.8 mg/kg) as a single intraperitoneal injection once daily for 3 days. Because of the lability of colchicine when exposed to light, dosing solutions were prepared daily from a stock solution of 50 mg/kg. Both stock solutions and dosing solutions were protected from light prior to injection.
Blood samples for determination of PRC were collected from the retro-orbital sinus at 18, 24, and 72 hours after the first injection. Because ether at concentrations that induce a surgical plane of anesthesia can induce increases in PRC,10 blood sampling was performed under a light ether anesthesia, that is, enough ether to render the animals manageable but not unconscious. Samples were taken within 3 to 5 minutes of exposure to ether, to further minimize the effect of ether on PRC.

Body weight and 24-hour urine volumes were determined prior to treatment and at 24-hour intervals thereafter. Based on the results of this experiment, a dose of 0.2 mg/kg colchicine once daily for 3 days was used in subsequent experiments.

To determine that alterations in PRC were not due to changes in renal renin concentration (RRC), renin was extracted from the kidneys of animals treated with saline, 0.2 or 0.4 mg/kg colchicine, and measured by radioimmunoassay (RIA) as described elsewhere.13 Protein was determined by the method of Lowry et al.12

The effect of colchicine on PRC following drug stimulation was determined according to the following protocol. Twenty-eight male Sprague-Dawley rats (weighing approximately 300 g) were divided into two groups of 14 each. One group received colchicine (0.2 mg/kg i.p.) once daily for 3 days; the control group received an equivalent volume of saline. Thirty minutes after injection of isoproterenol (25 mg/rat, i.p.) to stimulate renin secretion, the remaining rats in each treatment group received an equivalent volume of 0.9% saline. Solutions were prepared daily and protected from light.

Twenty-four hours after the last injection, half of each treatment group (seven rats) received isoproterenol (25 mg/rat, i.p.) to stimulate renin secretion. The remaining rats in each treatment group received an equivalent volume of saline. Thirty minutes after injection of isoproterenol or saline, rats were killed by decapitation and blood collected for determination of PRC. This experiment was repeated in a second group of 28 rats using furosemide (5 mg/kg, i.p.) as the stimulus to renin secretion.

Lumicolchicine was prepared from colchicine by exposure to ultraviolet light according to the method of Wilson and Friedkin.15 Rearrangement of colchicine to lumicolchicine, ethanol was removed by evaporation. A stock solution of 50 mg/ml lumicolchicine was prepared in 95% ethanol. Solutions for injection were prepared daily from this stock solution.

The effect of lumicolchicine on PRC was determined as previously described for colchicine. Lumicolchicine was administered to male Sprague-Dawley (250 to 300 g) rats at a dose of 0.2 mg/kg/day, i.p. Control rats received 0.9% saline. Isoproterenol (25 μg/rat, i.p.) was used as the stimulus to renin secretion and was administered as previously described. Blood samples were collected 30 minutes after isoproterenol for determination of PRC.

Urinary sodium concentration and hematocrit were determined in several animals from the control, colchicine (0.2 mg/kg), and lumicolchicine (0.2 mg/kg) groups, to monitor any drug effects on plasma volume that might cause an elevation in PRC. Urinary sodium concentration was determined by flame photometry and hematocrit by the micromethod.

Alterations in PRC can occur as a result of either changes in the secretion of renin or in changes in the metabolism of renin by the liver. To determine whether colchicine altered hepatic clearance of renin, rats (4/group) were treated with 0.2 mg/kg of either colchicine or lumicolchicine once daily for 3 days. Controls received an equivalent volume of saline. On the day of the experiment, rats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.), the abdominal aorta was exposed by a midline abdominal incision, and a polyethylene cannula inserted for collection of blood samples.

After a 30-minute stabilization period, a 0.5 ml blood sample was collected. The renal artery and vein leading to each kidney were clamped and a T1 blood sample (0.5 ml) taken immediately. Blood samples were also taken at 3, 5, 10, 20, and 45 minutes after clamping. The PRC was determined in each sample, and disappearance curves and half-life (t1/2) calculated.

Sample Handling

Blood samples were collected in chilled tubes containing ethylene diaminetetraacetic acid (EDTA, 1 mg/ml), centrifuged at 4°C, and plasma removed and stored frozen until determination of PRC. The PRC was determined by RIA for angiotensin I as previously described,13,14 after incubation of nonacidified plasma with 48-hour nephrectomized rat plasma containing excess angiotensinogen that was renin- and angiotensin-free.

Statistical Analysis

Data were analyzed by Student’s t test for unpaired data or analysis of variance. When analysis of variance was used, differences between experimental means were determined by the Student-Newman-Keuls test. Linear regression analysis was used for hepatic clearance experiments. In all cases, the probability level of 0.05 was used as the criterion of significance.

Results

Colchicine (0.2 mg/kg) had no effect on basal PRC, body weight, or 24-hour urine volume (table 1). Animals appeared healthy and showed no signs of dehydration or diarrhea. The increase in PRC in control animals during the 72 hours was not significant. Colchicine at 0.4 mg/kg caused a significant elevation of PRC at 72 hours and resulted in a decrease in body weight, which was evident at 48 hours, as well as diarrhea and dehydration, as determined by tenting of the skin. There was also a progressive decrease in 24-hour urine volume. The highest dose of colchicine (0.8 mg/kg/day) produced effects similar to those observed at 0.4 mg/kg/day (table 1). Diarrhea and dehydration...
TABLE I. Dose-Related Effects of Colchicine in Five Rats

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Plasma renin concentration (ng/ml/hr)</th>
<th>Body weight (g)</th>
<th>Urine volume (ml/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreatment</td>
<td>18 hrs</td>
<td>72 hrs</td>
</tr>
<tr>
<td>Saline</td>
<td>4.83 ± 1.19</td>
<td>5.71 ± 1.71</td>
<td>8.21 ± 0.94</td>
</tr>
<tr>
<td>Colchicine 0.2</td>
<td>4.28 ± 1.26</td>
<td>4.06 ± 0.64</td>
<td>7.77 ± 0.20</td>
</tr>
<tr>
<td>Colchicine 0.4</td>
<td>5.53 ± 1.10</td>
<td>6.26 ± 1.50</td>
<td>11.11 ± 0.46</td>
</tr>
<tr>
<td>Colchicine 0.8†</td>
<td>4.50 ± 0.91</td>
<td>9.01 ± 1.58</td>
<td>13.30* †</td>
</tr>
<tr>
<td></td>
<td>± 1.58</td>
<td>± 0.86</td>
<td>± 6</td>
</tr>
</tbody>
</table>

*Significantly different from saline-treated animals at the same time period.
†Significantly different from pretreatment values.
‡One of five animals died by 72 hours posttreatment.
Values are means ± SEM.

were more pronounced, and one animal died prior to the final observation. At 72 hours, PRC was 161% higher than that in control animals. Body weight and 24-hour urine volume were also decreased by 0.8 mg/kg/day colchicine. The RRC (ng AL/hr/mg protein) was unaltered by treatment with either 0.2 or 0.4 mg/kg colchicine (control, 118 ± 13; colchicine 0.2 mg/kg, 126 ± 21; colchicine 0.4 mg/kg, 97.4 ± 6).

Following 3 days of treatment with colchicine (0.2 mg/kg/day), basal PRC was unaltered (fig. 1). Furosemide (5 mg/kg, i.p.) caused a significant increase in PRC in saline-treated animals. In colchicine-treated rats, however, the effect of furosemide on PRC was inhibited.

When isoproterenol (25 µg/rat, i.p.) was used as the stimulus to renin secretion, similar results were observed (fig. 2). PRC was unaltered by colchicine (0.2 mg/kg) treatment alone as were urinary sodium concentration and hematocrit (table 2). Isoproterenol caused a significant increase in PRC in saline-treated animals. Although in colchicine-treated rats isoproterenol produced a significant increase in PRC, the magnitude of the response was blunted when compared to the control animals (fig. 2).

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**Figure 1.** Effect of 3 days of treatment with colchicine on plasma renin concentration (PRC) 30 minutes after furosemide stimulation. Values represent the mean ± SEM; * indicates significant difference from saline treatment; † indicates significant difference from furosemide treatment in control animals (n = 7).

**Figure 2.** Effect of 3 days of treatment with colchicine on plasma renin concentration (PRC) 30 minutes after isoproterenol stimulation. Values represent the mean ± SEM; * indicates significant difference from saline treatment; † indicates significant difference from isoproterenol treatment in control animals (n = 7).
TABLE 2. Effect of Colchicine and Lumicolchicine on Urinary Sodium Concentration and Hematocrit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No.</th>
<th>Urinary sodium (mEq/liter)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>10</td>
<td>139 ± 21</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.2</td>
<td>10</td>
<td>164 ± 42</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Lumicolchicine</td>
<td>0.2</td>
<td>9</td>
<td>126 ± 12</td>
<td>44 ± 2</td>
</tr>
</tbody>
</table>

Like colchicine, lumicolchicine at 0.2 mg/kg/day had no effect on basal PRC (fig. 3), body weight gain (data not displayed), hematocrit, or urinary sodium concentration (table 2). Isoproterenol caused a significant increase in PRC in saline-treated animals and this stimulation of PRC was not altered by prior treatment with lumicolchicine (fig. 3). Similar results were observed at a lumicolchicine dose of 0.4 mg/kg/day (data not displayed).

Hepatic clearance of renin showed a typical biphasic pattern15-16 with a rapid early clearance followed by a prolonged secondary phase, which was similar in all groups. The half-life of renin during the early phase (0-10 minutes) was not different among the three treatment groups (control 12.46, colchicine 9.25, lumicolchicine 10.85 minutes). Similarly, the $t_{1/5}$ of the secondary phase (10 to 45 minutes) was similar in all groups (control 37.7, colchicine 39.7, lumicolchicine 44.7 minutes).

Discussion

In most secretory systems, the effect of antimitotic alkaloids has been to decrease secretion.1-5 Colchicine and the vinca alkaloids, however, have been reported to both stimulate8,9 and suppress the secretion of renin.4,6 The inconsistency of these results may be related in part to the drug dose or to the preparation used to study renin secretion. In the present study, PRC was used as an indication of renin release. While a dose-related effect of colchicine on basal PRC was observed (table 1), rats treated with the highest doses (0.4 and 0.8 mg/kg/day) had a significant weight loss and diarrhea and decrease in urine output. These latter findings indicate that the animals were volume-depleted and, therefore, make the interpretation of the changes in PRC difficult. The rise in PRC may well be related to the observed toxic effects of the drug, which may cause volume depletion,9 however, the possibility that colchicine has a direct stimulatory effect on renin secretion cannot be excluded.

The animals receiving the lowest dose of colchicine (0.2 mg/kg) did not demonstrate any changes in weight, urine volume, sodium concentration, or hematocrit and basal PRC (tables 1 and 2), or RRC. This same dose of colchicine, however, inhibited both isoproterenol- and furosemide-induced increases in PRC (figs. 1 and 2).

The present results are consistent with the in vitro observations of Capponi and Valloton9 who demonstrated that vincristine had no effect on basal renin secretion in kidney slices from control or adrenalectomized rats but that renin release was inhibited following exposure to isoproterenol. These data, however, do not answer the question of how a colchicine effect on PRC is mediated. A well-known pharmacological action of colchicine is its ability to bind to tubulin.17 This binding alters the tubulin-microtubule equilibrium and results in depolymerization of cytoplasmic microtubules. This action of colchicine has been associated with altered secretion in several systems.3,5

The colchicine-tubulin binding reaction in vivo is a slow process that does not reach equilibrium for 6 to 8 hours.17 Thus, by using a low dose, (0.2 mg/kg) over a 3-day period, the likelihood of colchicine acting on the tubulin-microtubule system was enhanced, especially since the side effects of weight loss and diarrhea associated with higher doses of colchicine were absent.

Lumicolchicine (fig. 3) produced no alteration of either basal or stimulated PRC. Unlike colchicine, lumicolchicine does not interact with tubulin although it does share other pharmacological effects with colchicine.18 Furthermore, neither colchicine nor lumicolchicine altered the hepatic clearance of renin, suggesting that colchicine acts on secretion rather than metabolism of renin. Palaic18 recently demonstrated that $10^{-5}$M colchicine could decrease the responsiveness of isolated guinea pig aorta to angiotensin II. The decreased responsiveness was attributed to a decrease in angiotensin receptors caused by interruption by colchicine of the flow of newly synthesized receptors to the
surface. If colchicine produces similar phenomena in vivo, excess plasma angiotensin would feed back and inhibit renin release from the kidney. The concentration of angiotensin II was not determined in our present study; however, it is unlikely that an effect on angiotensin receptors is responsible for the results observed in this study since neither basal PRC nor angiotensin I concentration in plasma (data not shown) were altered by treatment with colchicine (0.2 mg/kg) or lumicolchicine (0.2 mg/kg). The effects observed in these studies suggest, therefore, that altered PRC in response to drug stimulation following colchicine treatment may be related to interaction of colchicine with the tubulin-microtubule system.

Hackenthal found that colchicine \((5 \times 10^{-5} \text{ M})\) did not alter basal or isoproterenol-stimulated renin secretion whereas vinblastine \((5 \times 10^{-3} \text{ M})\) inhibited both basal and stimulated secretion. The lack of a colchicine effect in this study may be due to the lower antimicrotubule potency of colchicine compared to vinblastine and the slower tubulin-binding reaction of colchicine compared to vinblastine. The results presented herein are similar to findings observed in other secretory systems. In isolated pancreatic islets, exposure to colchicine caused a progressive decrease in insulin secretion while no effect was observed in the same preparation following lumicolchicine treatment. Colchicine has also been shown to inhibit retinol-binding protein release from the liver and milk secretion from rat mammary glands. In these cases, disruption of cytoplasmic microtubules was suggested as the cause of the functional inhibition. In the case of milk secretion, ultrastructural findings supported the functional observation.

In summary, we have shown that doses of colchicine above 0.2 mg/kg caused an increase in PRC which could be related to side effects of the drug. A colchicine dose of 0.2 mg/kg/day was without overt toxicity and had no effect on basal renin secretion. At this dose of colchicine (0.2 mg/kg), however, renin secretion following stimulation with either furosemide or isoproterenol was significantly inhibited. Furthermore, lumicolchicine, which lacks antimicrotubule effects, had no effect on either basal or stimulated renin secretion. These data suggest that a microtubule component may be involved in renin secretion.

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