Effects of indomethacin on renal hemodynamics, urinary enzymes and thromboxane B₂ following envenomation of Russell's viper in dogs.

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The effects of indomethacin (5 mg/kg, i.v.) on renal hemodynamics, urinary enzymes, plasma and urinary thromboxane B₂ (TXB₂) following a 40 min intrarenal arterial infusion of Russell's viper venom (RVV, 1.25 µg/kg/min) were studied in anesthetized male mongrel dogs. All parameters were determined before and at every 20 min after envenomation. Urinary enzymes were assayed by colorimetric method. Plasma and urinary thromboxane B₂ (P₄₅ₓ₅₅, U₄₅ₓ₅₅) were measured by radioimmunoassay after extraction and purification steps. The reduction of mean arterial pressure (MAP) and the increase of urine flow rate (V), P₄₅ₓ₅₅ and U₄₅ₓ₅₅ induced by RVV were inhibited by indomethacin pretreatment. Renal blood flow (RBF) and glomerular filtration rate (GFR), relatively unchanged by RVV, were markedly reduced by indomethacin pretreatment. Only urinary N-acetyl-β-D-glucosaminidase (NAG) was increased after envenomation. These results suggest an augmented synthesis of TXB₂ and/or disturbance of active tubular reabsorption of U₄₅ₓ₅₅. After the inhibition of prostaglandin synthesis by indomethacin, the reduction of renal function and hypertensive response to RVV may be accounted for by the renin-angiotensin system. The elevation of U₄₅ₓ₅₅ indicates a direct nephrotoxicity of RVV.

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คณะครูสังกัดได้ศึกษาถึงเรื่องของพิษหมากเหงาที่ก่อการการสัมผัสของไขเป็นเวลา 40 นาที ในขณะ
1.25 ในกรังราว/กิโลเมตร/นาที ที่มีการกระทำของโครงการ คือ เลี้ยงขี้มีนในที่สูง และระคายกรักกิจการ บุรุษ
ในสภาพยา และบุรุษของสุนัขที่ใช้เป็นการฉีกต่อเนื่องในกระเพาะที่มีการเคาะที่ 4 กลับกับกิโลกรัม
(7 ตัว) ต่อการฉีกที่ 40 นาที เปรียบเทียบกับสุนัขที่ไม่ได้รับยาในกระเพาะที่มากกว่า (7 ตัว)
โดยวิธีทดลอง
ทุก ๆ 20 นาที ภายหลังการเป็นการฉีกแล้ว รักษาสุนัขที่มีใบยาในกระเพาะต่อเนื่องในการวิจัยวิธีต่อเนื่องมี
ระดับ ครบกิจการ บุรุษ ในสภาพยา และบุรุษของวิทยาการฉีกต่อเนื่องไม่ได้แสดงถึงความสำคัญของ
การทำให้สุนัขแข็ง ที่พิษหมากเหงาทำให้ความเสี่ยงต่อกองที่ เพื่อการเข้าสู่กระเพาะและเพื่อระคายกรักกิจการ
บุรุษ ในสภาพยา และบุรุษของวิทยาการฉีกต่อเนื่องไม่ได้แสดงถึงความสำคัญของไขเป็นเวลา 40 นาที เปียกที่ไป
ยังโดยไม่ได้ต่อเนื่อง การทำให้สุนัขต่อเนื่องไม่ได้เป็นการฉีกต่อเนื่องเลย โดยไม่ได้รับยาใน
กระเพาะ เลี้ยงขี้มีนในภาพจะมีการกระทำที่ต่อเนื่องแล้วต่อเนื่องกับการกระทำที่สุนัขได้รับยาใน
กระเพาะ (สุนัข) ที่ไม่ได้เป็นการฉีกต่อเนื่องมากกว่า
เหลือจากนี้การสัมผัสของไขเป็นเวลา 40 นาที เผื่นขึ้น
และ/หรือมีการกระทำอย่างต่อเนื่องกลับย้อน กระเพาะเท่าที่มี บุรุษ ที่ทำให้การพิษหมากเหงาไปสู่
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Russell's viper venom (RVV) is known to cause renal damage including tubular necrosis, cortical necrosis, and glomerulonephritis. Acute renal failure (ARF) which is recognized to be an important cause of death in patients bitten by Russell's viper may partly result from tubulotoxicity of this venom. It has been suggested that a wide variety of enzymes normally excreted into the urine are increased in various disease states including in renal tubular cell injury. The early work of Wellwood et al. had indicated that certain lysosomal enzymes from the proximal tubular cells: N-acetyl-beta-D-glucosaminidase (NAG) and beta-galactosidase (GAL), or from the brush border: gamma-glutamyl-transpeptidase (GGT) and alanine-aminopeptidase (AAP) are early markers of tubular damage induced by aminoglycosides. There was no data on enzymuria despite clinical evidence suggesting a direct tubulotoxicity of RVV.

Furthermore, the reduction in renal hemodynamics clearly observed in experimental dogs envenomated with RVV, may also play a role in the initial pathogenesis, in the ischemic model of ARF. It has been proposed that catecholamine release, renin-angiotensin activation, and prostaglandin release may be responsible for the impairment of renal function.

In this study the effects of Russell's viper venom on renal hemodynamics, urinary enzymes, plasma thromboxane B2 (P<sub>TxB2</sub>) and urinary thromboxane B2 (U<sub>TxB2</sub>) in dogs pretreated with indomethacin compared to non-pretreated dogs were investigated in an attempt to elucidate the relationship between the nephrotoxicity of RVV and urinary enzymic activities, the role of thromboxane B2 in renal hemodynamic changes and effects of indomethacin, a cyclooxygenase inhibitor.

### Material and Method

Fourteen mongrel dogs weighing 10 to 15 kg were anesthetized by intravenous injection of thiopentone sodium at the dosage of 20-25 mg/kg. Supplementary doses were given as necessary to maintain light anesthesia. A femoral artery was cannulated for arterial blood sample collection and for recording of arterial blood pressure and heart rate. The heart rate was recorded on a Grass physiograph. Renal artery was cannulated for venom infusion. Femoral vein was cannulated for fluid infusion. A polyethylene catheter was introduced into the bladder for urine collection.

Renal plasma flow and glomerular filtration rate were measured by p-aminohippurate and inulin clearances using standard techniques. Renal blood flow was calculated from p-aminohippurate clearance and packed red cell volume. Plasma levels of p-aminohippurate and inulin were maintained approximately at 0.03 mg/ml and 0.20 mg/ml by sustained infusion of isotonic saline solution containing p-aminohippurate (Merck, Sharpe and Dohme) and inulin (Sigma Chemical Co.) following the priming doses. After 40 min of equilibration, urine was collected for 20 min and an arterial blood sample was obtained at the midpoint of urine collection. This served as the control period. Thence, Russell's viper (Vipera russelli siamensis) venom, donated by the Science Division of the Thai Red Cross Society, was given by intrarenal arterial infusion at a dose of 0.05 mg/kg. The lyophilized venom was dissolved in 20 ml of 0.9% sodium chloride solution (NSS). The infusion rate was 0.5 ml/min.

For the indomethacin pretreated group, indomethacin was administered intravenously at a dose of 5 mg/kg, 40 min before envenomation of RVV. Blood sampling and urine collection were made twice over a period of 40 min, after indomethacin pretreatment and at every 20 min after envenomation.

The analysis of urine and blood samples were performed of p-aminohippurate and inulin, sodium and potassium (flame photometric method), chloride (by chloridometer), TxB2 (as shown in diagram I). Urinary lysosomal enzyme activities were measured by colorimetric method as shown stepwise in diagram II.

### Diagram I Steps of TxB2 assay.

- **Sample**
  - Centrifugation (3000 rpm, 5 min)
- **Supernatant**
  - Extraction (with chloroform)
  - Purification (by silicic acid column)
  - RIA
    - (TxB<sub>2</sub>(H) RIA Kit supplied by NEN)
Diagram II Steps of analysis of urinary enzyme activities.

Result

The reduction in arterial blood pressure after envenomation was inhibited by indomethacin pretreatment. Heart rate was slightly decreased in both groups after envenomation (Table 1.) Packed red cell volume was not altered by indomethacin pretreatment but was slightly increased in response to RVV. Renal blood flow (RBF) and renal plasma flow (RPF) were slightly increased during the first 20 min after envenomation but gradually decreased toward the control level, while indomethacin caused a significant decrease in RBF and RPF with further decreases after envenomation. Glomerular filtration rate (GFR), relatively unchanged by RVV alone, was decreased slightly after indomethacin pretreatment and significantly following envenomation. The rising of urine flow rate (V) by 15-65% in response to RVV was inhibited by indomethacin pretreatment (Fig. 1). Urinary excretion of sodium (Un Na) V, chloride (U Cl V) and potassium (U K V), and the fractional excretion of sodium (FE Na) and chloride (FE Cl) were increased during the first 20 min of envenomation parallel to the increase in urine flow rate and then declined toward the control level. Indomethacin pretreatment tended to decrease the urinary excretion of sodium, chloride and potassium which gradually increased toward the control level during a 40-min of envenomation. Fractional excretion of sodium, chloride and potassium rose throughout the experimental period but not significantly different from the control (Table 2). The urinary enzyme activity of NAG, significantly increased after the end of a 40-min intrarenal arterial infusion of RVV, was elevated slightly by indomethacin pretreatment and again significantly during envenomation. Other urinary enzyme activities fluctuated but showed a tendency toward an increase after envenomation (Fig. 2). A 7 to 10 fold elevation of urinary TxB2 was observed during envenomation. Plasma TxB2 followed a parallel elevation. Indomethacin completely inhibited the rise in P TxB2 and U TxB2 (Fig. 3, 4).

Table 1 Effect of Russell's viper venom on blood pressure and heart rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Indomethacin</th>
<th>Russell's viper venom</th>
<th>NSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>40 min</td>
<td>20 min</td>
<td>40 min</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>128 ± 8</td>
<td>not given</td>
<td>118 ± 10**</td>
<td>118 ± 12</td>
</tr>
<tr>
<td></td>
<td>122 ± 24</td>
<td>128 ± 22*</td>
<td>130 ± 19*</td>
<td>137 ± 17*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>154 ± 11</td>
<td>not given</td>
<td>147 ± 9</td>
<td>135 ± 10</td>
</tr>
<tr>
<td></td>
<td>145 ± 21</td>
<td>130 ± 17</td>
<td>128 ± 15</td>
<td>130 ± 25</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 7

*P<0.05, **P<0.025, † P<0.01 when compared to the control

NSS = Normal saline solution
Figure 1 Effects of Russell's viper venom on packed cell volume (PCV), renal plasma flow (RPF), renal blood flow (RBF), glomerular filtration rate (GFR) and urine flow rate (V) in indomethacin non-pretreated (n=7) and pretreated dogs (n=7). Values are mean ± S.D., *P<0.05, †P<0.001 when compared to the control. NSS = normal saline solution.
Table 2 Effect of Russell's viper venom on sodium, chloride and potassium excretion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Indomethacin</th>
<th>Russell’s viper venom</th>
<th>NSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 min</td>
<td>40 min</td>
<td>20 min</td>
</tr>
<tr>
<td>( U_{Na} ) V (mEq/min)</td>
<td>40.64 ± 40.52</td>
<td>not given</td>
<td>not given</td>
<td>53.34 ± 36.02</td>
</tr>
<tr>
<td>( F_{Na} ) (%)</td>
<td>1.81 ± 2.17</td>
<td>not given</td>
<td>not given</td>
<td>3.25 ± 3.19*</td>
</tr>
<tr>
<td>( U_{Na} ) V (mEq/min)</td>
<td>48.4 ± 32.48</td>
<td>38.09 ± 41.14</td>
<td>35.85 ± 38.22</td>
<td>35.75 ± 25.88</td>
</tr>
<tr>
<td>( F_{Na} ) (%)</td>
<td>2.2 ± 1.1</td>
<td>2.2 ± 1.1</td>
<td>3.3 ± 4.2</td>
<td>14.9 ± 30.1</td>
</tr>
<tr>
<td>( U_{Cl} ) V (mEq/min)</td>
<td>45.85 ± 45.62</td>
<td>not given</td>
<td>not given</td>
<td>59.36 ± 39.13</td>
</tr>
<tr>
<td>( F_{Cl} ) V (%)</td>
<td>2.32 ± 2.86</td>
<td>not given</td>
<td>not given</td>
<td>4.33 ± 4.76*</td>
</tr>
<tr>
<td>( U_{Cl} ) V (mEq/min)</td>
<td>54.2 ± 50.12</td>
<td>51.19 ± 68.02</td>
<td>43.57 ± 65.71</td>
<td>46.31 ± 43.48</td>
</tr>
<tr>
<td>( F_{Cl} ) V (%)</td>
<td>2.95 ± 2.25</td>
<td>3.4 ± 2.7</td>
<td>5.1 ± 8.0</td>
<td>5.2 ± 5.5</td>
</tr>
<tr>
<td>( U_{K} ) V (mEq/min)</td>
<td>11.25 ± 4.85</td>
<td>not given</td>
<td>not given</td>
<td>12.54 ± 4.54</td>
</tr>
<tr>
<td>( F_{K} ) (%)</td>
<td>23.53 ± 15.05</td>
<td>not given</td>
<td>not given</td>
<td>32.16 ± 17.05*</td>
</tr>
<tr>
<td>( U_{K} ) V (mEq/min)</td>
<td>8.74 ± 7.07</td>
<td>6.25 ± 3.65</td>
<td>6.48 ± 4.07</td>
<td>6.65 ± 3.89</td>
</tr>
<tr>
<td>( F_{K} ) (%)</td>
<td>18.45 ± 8.1</td>
<td>17.4 ± 9.2</td>
<td>25.3 ± 19.2</td>
<td>27.7 ± 18.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 7

UV = urinary excretion, FE = fractional excretion, Na = sodium, Cl = chloride, K = potassium.

*P < 0.05 when compared to the control.
Figure 2  Effects of Russell’s viper venom on urinary enzyme activities (n = 7). Values are mean ± S.D., *P < 0.05 when compared to the control. NSS = normal saline solution.
Figure 3: Effects of Russell’s viper venom on the urinary and plasma levels of thromboxane B₂ in the indomethacin non-pretreated (n = 7) and pretreated dogs (n = 7). Values are mean ± S.D., *P < 0.05, **P < 0.025, † P < 0.01, ***P < 0.005, †† P < 0.001. When compared to the control. NSS = normal saline solution.
Figure 4 Comparing of the percentage changes from the control level of the plasma and urinary thromboxane B₂ (P₂TXB₂ and U₂TXB₂) between the indomethacin non-pretreated □ and indomethacin pretreated ■ dogs after 20 min and 40 min of envenomation and a 20-min infusion of normal saline solution (NSS). Each bar is mean ± S.D. of 7 dogs.
Discussion

The results from our studies show that Russell's viper venom directly infused via renal artery at the dose of 1.25 μg/kg/min can slightly affect the systemic circulation and renal hemodynamics. The reduction of mean arterial blood pressure may be attributable to splanchnic vasodilatation. It is believed that Russell's viper venom causes vasodilatation through its effect on prostaglandins and kinin release as well as other slow reacting substances. Our results showed a parallel increase of \( P_{\text{TXB}_2} \) and \( U_{\text{TXB}_2} \) due to Russell's viper venom despite no marked change in hemodynamics. The increased \( P_{\text{TXB}_2} \) and \( U_{\text{TXB}_2} \) may have partly been the result of augmented synthesis of \( \text{TXB}_2 \) in the renal vasculature. The vasoconstrictor action of \( \text{TXB}_2 \) may be counteracted by other renal hormones including vasodilatory prostaglandins. Indomethacin, a cyclooxygenase inhibitor, given prior to envenomation can completely inhibit the rise in \( P_{\text{TXB}_2} \) which accompanied the marked increase in blood pressure in response to the venom. Activation of renin-angiotensin system may be responsible for the hypertensive effect of the venom since, in other study, angiotensin converting enzyme inhibitor has been shown to attenuate this effect. The slight elevation of packed cell volume in both groups was presumably due to splenic contraction. Although the renal blood flow and glomerular filtration rate were relatively unchanged after envenomation, it should be noted that urine flow rate was increased by approximately 15-65%, accompanied by an increase in urinary excretion of sodium, chloride, potassium and thromboxane \( \text{B}_2 \) as well. They could be accounted for by a direct tubular dysfunction since thromboxane \( \text{B}_2 \), like uric acid, is actively reabsorbed from the proximal tubule.

Recently it has been reported that Russell's viper venom induced depolarization of the proximal tubular cell possibly by 3 mechanisms. It has been proposed that the failure of the proximal tubular reabsorption of sodium giving rise to a sodium overload in the distal tubular urine can induce the secretion of renin by the macula densa. The activation of angiotensin could induce a decrease in the renal blood flow and cortical ischemia followed by a decrease in glomerular filtration rate. The reduction in renal blood flow may be modulated by the production of vasodilatory prostaglandins, \( \text{PGI}_2 \) and \( \text{PGE}_2 \), in the kidney. We did not detect any production of \( \text{PGI}_2 \) and \( \text{PGE}_2 \) but clearly observed a decreased renal blood flow and glomerular filtration rate in response to Russell's viper venom after inhibition of prostaglandin synthesis by indomethacin. The activation of renin-angiotensin system could likely account for this. Our result may have reflected the role of angiotensin II on prostaglandin production which McGiff et al were the first to demonstrate and was subsequently confirmed by many investigators. Urine flow rate changes similar to renal blood flow and glomerular filtration rate during the first 20 min of envenomation then rose despite the decrease in renal blood flow and glomerular filtration rate. Urinary excretion of sodium, chloride and potassium seemed to have decreased relatively less than the decrease in glomerular filtration rate and therefore the fractional excretion of sodium, chloride and potassium were increased. This result suggested that the inhibition of tubular reabsorption of sodium presumably occurred at sites proximal to the distal nephron thus a definite amount of sodium reached this part of the nephron and a small amount under reabsorption in exchange to potassium. Aldosterone, responsible for sodium reabsorption in the distal nephron, may be increased primarily due to the increase in angiotensin II. The correlation between plasma aldosterone concentration and plasma renin activity has been described by Zadik and Kowarski that the decrease in plasma renin activity is most likely the primary cause of the lower plasma aldosterone concentration; possibly, the opposite may be true. Elevated level of NAG probably indicated the evidence of renal injury corresponding to other types of renal disease or injury previously reported. The measurement of AAP, γ-GT or β-GAL appeared on the contrary, to have had little significance. In summary, Russell's viper venom at this dose may directly alter the active reabsorption in the tubular cells. Elevation of \( P_{\text{TXB}_2} \) and \( U_{\text{TXB}_2} \) indicated the augmentation of synthesis and/or alteration of tubular reabsorption. Activation of the renin-angiotensin system seemed to have been responsible for the decrease in the renal blood flow and glomerular filtration rate. Experimental data on plasma renin activity changes should be worked out. It remains to be determined whether the levels of prostacycline (\( \text{PGI}_2 \)), \( \text{PGE}_2 \) and aldosterone are changed and if so, what role they may eventually play in renal hemodynamic changes. Renal histological changes should be performed in accompaniment with a study on urinary enzymes.
Acknowledgement

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References

16. Huang HC. Effects of phospholipase A2 from Vipera russelli venom on blood pressure, plasma prostacyclin level and renin activity in rats. Toxicol 1984; 22 (2) : 254-264
