ANTICOAGULANT ACTIVITY OF ACENOCOUMARIN
IN EXPERIMENTAL ANIMALS

By
M.N. JINDAL AND D S. SHAH*

From the Department of Pharmacology, B. J. Medical College, Ahmedabad
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Anticoagulant activity of acenocoumarin (Sintrom) has been determined in dogs. Prothrombin values were determined according to modified Quick's method (Montigel 1962). Prothrombin index and coagulation valency were deduced from the prothrombin time. The results were compared with the reference drug ethylbiscoumacetate. Acenocoumarin was found to be 60 times more potent than ethylbiscoumacetate. The onset of action was however slower but the total duration of action was much longer than ethylbiscoumacetate. There was no evidence of toxic symptoms in the dogs even in high doses. The mechanism of anticoagulant action seems to be hypoprothrombinemia caused by the drug.

Acenocoumarin is a recently developed new anticoagulant drug of the 4 hydroxycoumarin series. Most of the compounds of these series are known to produce their anticoagulant effect in man by depressing the formation of prothrombin by the liver, thereby affecting the prothrombin time, prothrombin index and coagulation valency. Qualitatively, the effect of most of the coumarin derivatives on the coagulation mechanism is the same but there is marked variation in the quantitative values of the different compounds. Bishydroxy-coumarin is a potent depressant of the prothrombin values but the absorption and excretion of the drug is markedly variable. Its onset of action is slow but the effect lasts for a longer time. On the other hand ethylbiscoumacetate produces a rapid onset of action but the effect fades away too soon. Acenocoumarin has been claimed to produce an intermediate effect between these two extremes (Norwich 1959, Pratt 1956). Available literature on clinical and experimental experience with acenocoumarin shows that little work has been done on its antiprothrombin value in laboratory animals.

It was, therefore, thought proper to investigate this drug on some suitable laboratory animals for this purpose, and to assess its exact place amongst the anticoagulants regarding onset, duration and mechanism of its action. Most of the workers have used rabbits to determine the prothrombin values

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ACENOCOUMARIN
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A.H.
Colledge, Ahmedabad

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yl biscoumacetate. The onset of 
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s to be hypoprothrombinemia 

The first and the second group of animals were administered acenocou-
arin and ethylbiscoumacetate respectively in doses equivalent to the normal 
adult human dose. Accordingly, acenocoumarin was given in a dose of 0.5 
mg/kg body weight and ethylbiscoumacetate in a dose of 30 mg/kg body 
weight. The drugs were administered once only and prothrombin estimations 
were done once daily till prothrombin level returned to their normal 
values.

Animals in group 3, 4 and 5 were given acenocoumarin in graded doses 
i.e. 0.4 mg/kg, 0.8 mg/kg, and 1.2 mg/kg, of body weight respectively. The 
drug was again administered in one single dose and prothrombin estimations 
were done daily till the values touched the normal.

Animals in group 6 and 7 were given acenocoumarin in doses of 0.4 
mg/kg, and 0.6 mg/kg body weight respectively. In these animals the drug 
was given daily on 3 consecutive days. Prothrombin estimations were done 
daily, till normal values were achieved after stopping the drug.

Method for estimation of prothrombin value.—The drug was administered 
between 3 to 4 p.m. of the day, while the blood sample for estimation of 
prothrombin time was taken between 9 to 10 a.m. in the next morning. The 
prothrombin time was measured according to modified Quick's one stage 
method as follows. 1.6 ml of blood was taken in a sterile syringe containing 
0.4 ml of isotonic sodium citrate (3%) solution, from the saphenous vein of 
the dog. The sample was then centrifuged at 1700 r.p.m. for 7 min, and
thus plasma was separated from the deposit of red cells. The sample was kept in the refrigerator (5°C) till the test was performed. In the thick walled test tube one tablet of thrombokinase (Geigy) with calcium was carefully crushed to a fine powder with a glass rod. Two drops of distilled water were added and stirred with the powder to form a smooth paste. To this was added 2.5 ml of distilled water and the contents of the tube were thoroughly mixed. The suspension was then warmed in an incubator to 37.0 ± 0.5°C for 15 min. 0.1 ml of plasma was placed in a test tube and was warmed to a temperature of 37.0 ± 0.5°C in an incubator for 2 min. 0.2 ml of the suspension was then added to this test tube and at the same moment a stop watch was started. The time that elapsed before the plasma solidified was measured. Three such readings were taken with each sample of plasma, and the mean of the 3 was taken as the prothrombin time.

After measuring the normal prothrombin time of the animal in the morning, the drug uniformly mixed with milk was given in the afternoon. Prothrombin time was converted into prothrombin index and prothrombin percentage (coagulation valency) with the help of Geigy’s prothrombinometer.

RESULTS

The results of the anticoagulant activity of acenocoumarin have been illustrated in Table No. I, group I to VI (also Fig. 1).

It is evident that acenocoumarin when given in comparative human therapeutic doses, showed maximum hypoprothrombinemic action after 42 hrs, the action was maintained for 24 hrs, thereafter it started receding and was back to normal in 48 to 72 hrs With ethylbiscoumacetate, the peak action was achieved in 18 to 24 hrs, was maintained for 24 hrs and it returned to normal within 24 to 48 hrs.

The effect of acenocoumarin in graded doses has been illustrated in Table I, groups II,III and IV. It is evident from these results thatacenocoumarin has shown a quantitative response when administered in graded doses ranging as 0.4 mg/kg, 0.8 mg/kg, 1.2 mg/kg body weight respectively,

The animals in group VI and VII were given acenocoumarin in doses of 0.4 mg and 0.6 mg/kg body weight respectively on three consecutive days. The peak of cumulative action was seen after 66 hrs of the first dose. It was maintained from 48 to 72 hrs after stopping the drug, thereafter, the effect started declining and the normal values were achieved within 48 to 72 hrs.
cells. The sample was
formed. In the thick walled
tube and was warmed to a
min. 0.2 ml of the suspen-
solidified was measured.

of plasma, and the mean

were thoroughly

incubator to 37.0 ± 0.5°C

were thoroughly

suspended moment a stop watch

of Geigy’s prothrombin-

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macetate, the peak action

has been illustrated in

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acenocoumarin in doses of

of the first dose. It was

rug, thereafter, the effect


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\begin{array}{|c|c|c|c|c|}
\hline
\text{Drug & dose.} & \text{Time in hrs after administration of drug} & \text{Mean prothrombin time in sec} & \text{Standard deviation of the mean} & \text{Prothrombin Index} & \text{Coagulation valency} \\
\hline
\text{Acenocoumarin} & 0 & 12 & ± 0.223 & 100 & 100 \\
0.5 mg/kg body wt & 18 & 14 & ± 0.2937 & 85.7 & 71 \\
& 42 & 23 & ± 2.937 & 52.1 & 24 \\
& 66 & 23 & ± 1.837 & 52.1 & 24 \\
& 90 & 15 & ± 1.118 & 80 & 61 \\
& 114 & 14 & ± 0.2091 & 85.7 & 71 \\
& 138 & 12 & ± 2.121 & 100 & 100 \\
\hline
\text{Ethylbis-coumacetate} & 0 & 12 & ± 2.121 & 100 & 100 \\
30 mg/kg body wt & 18 & 20.3 & ± 1.908 & 59.1 & 31 \\
& 42 & 21 & ± 1.458 & 57.1 & 29 \\
& 66 & 15 & ± 1.904 & 80 & 61 \\
& 90 & 12 & ± 1.225 & 100 & 100 \\
\hline
\text{Acenocoumarin} & 0 & 13 & ± 2.025 & 100 & 100 \\
0.4 mg/kg body wt & 18 & 14 & ± 2.846 & 93.1 & 85 \\
& 42 & 20 & ± 2.927 & 65 & 37 \\
& 66 & 21 & ± 1.690 & 62 & 34 \\
& 90 & 16 & ± 2.268 & 82.25 & 63 \\
& 114 & 14 & ± 2.927 & 93.1 & 85 \\
& 138 & 13 & ± 2.000 & 100 & 100 \\
\hline
\text{Acenocoumarin} & 0 & 14 & ± 1.964 & 100 & 100 \\
0.8 mg/kg body wt & 18 & 16 & ± 2.132 & 87.5 & 72 \\
& 42 & 25 & ± 3.189 & 56 & 28 \\
& 66 & 25 & ± 3.162 & 56 & 28 \\
& 90 & 22 & ± 2.927 & 63.5 & 36.5 \\
& 114 & 19 & ± 1.871 & 73.5 & 50.5 \\
\hline
\end{array}
\]

TABLE I
Showing the effect of acenocoumarin and ethylbiscoumacetate on prothrombin time, prothrombin index and coagulation valency
### TABLE I (Contd)

<table>
<thead>
<tr>
<th>Drug &amp; dose</th>
<th>Time in hr. after administration of drug</th>
<th>Mean prothrombin time in sec</th>
<th>Standard deviation of the mean</th>
<th>Prothrombin Index</th>
<th>Coagulation valency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>138</td>
<td>15</td>
<td>± 2.739</td>
<td>83.5</td>
<td>86</td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenocoumarin</td>
<td>0</td>
<td>15</td>
<td>± 2.689</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>18</td>
<td>± 2.017</td>
<td>83.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>34</td>
<td>± 2.283</td>
<td>44.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>44</td>
<td>± 3.209</td>
<td>34.5</td>
<td>9</td>
</tr>
<tr>
<td>1.2 mg/kg body wt</td>
<td>90</td>
<td>40</td>
<td>± 2.449</td>
<td>37.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>33</td>
<td>± 2.112</td>
<td>45.7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>18</td>
<td>± 2.257</td>
<td>83.3</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>15</td>
<td>± 2.738</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Group V</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenocoumarin</td>
<td>0</td>
<td>20</td>
<td>± 3.117</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>22</td>
<td>± 1.873</td>
<td>91</td>
<td>80</td>
</tr>
<tr>
<td>0.4 mg/kg body wt</td>
<td>42</td>
<td>25</td>
<td>± 2.989</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>60</td>
<td>± 4.955</td>
<td>33.3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>58</td>
<td>± 6.761</td>
<td>34.5</td>
<td>9</td>
</tr>
<tr>
<td>for three consecutive days</td>
<td>114</td>
<td>51</td>
<td>± 1.774</td>
<td>39.2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>34</td>
<td>± 2.112</td>
<td>58.8</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>26</td>
<td>± 1.868</td>
<td>75.4</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>186</td>
<td>20</td>
<td>± 3.384</td>
<td>100</td>
<td>100</td>
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<tr>
<td><strong>Group VI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenocoumarin</td>
<td>0</td>
<td>17</td>
<td>± 5.857</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>19</td>
<td>± 2.325</td>
<td>90</td>
<td>79</td>
</tr>
<tr>
<td>0.6 mg/kg body wt for three consecutive days</td>
<td>42</td>
<td>26</td>
<td>± 2.037</td>
<td>65</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>60</td>
<td>± 1.779</td>
<td>28.3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>60</td>
<td>± 3.783</td>
<td>28.3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>55</td>
<td>± 3.116</td>
<td>31</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>47.5</td>
<td>± 3.865</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>162</td>
<td>35</td>
<td>± 1.196</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>186</td>
<td>25.75</td>
<td>± 1.149</td>
<td>66</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>17</td>
<td>± 1.336</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*The results are calculated as mean of 5 observations in the 1st group and 8 observations in the remaining groups.*
The effect of acenocoumarin with a higher dose in group VII was proportionately greater than that observed in group VI and here recovery was after 72 to 96 hrs.

Thus it is evident that once the prothrombin index is markedly reduced, the time taken to reach the normal value is considerably delayed even though the drug may be stopped completely. This is illustrated in Table I and group V and VI.
During the course of these investigations not a single animal showed external bleeding from any part of the body.

**DISCUSSION**

In the present study, anticoagulant property of acenocoumarin has been investigated in dogs and the results have been compared with ethylbiscoumacetate, which was used as a reference drug for this purpose. The evaluation of results in this study was based on estimation of prothrombin level in blood from 00 hr to 214 hrs, after administration of a single as well as multiple doses of the anticoagulants. Prothrombin index and coagulation valency were determined from the prothrombin time. The coagulation valency desirable in clinical cases, whenever anticoagulant therapy is indicated, is between 10 to 30 percent of normal (Robson and Keele, 1956). In the present study, this level has been achieved by acenocoumarin, when administered in a single dose of 0.5 mg/kg body weight, after 42 hrs; while in the case of ethylbiscoumacetate, these values are achieved in a shorter time i.e. 18 to 24 hrs, but with a dose of 30 mg/kg body weight. The total duration of effect with acenocoumarin is however, much longer i.e. from 114 hrs to 138 hrs; while in case of ethylbiscoumacetate the effect passes off completely within 66 to 90 hrs of administration.

When gradually increasing doses of acenocoumarin were administered i.e. 0.4 mg/kg, 0.8 mg/kg and 1.2 mg/kg body weight, there was proportionately greater response, as regards prothrombin time, prothrombin index and coagulation valency. This indirectly suggests that the drug is proportionately absorbed from the gastro-intestinal tract. Besides, it is also evident that acenocoumarin is 60 to 75 times more potent than ethylbiscoumacetate (Fig. I).

In another series of experiments, when acenocoumarin was administered daily in a dose of 0.4 mg/kg and 0.6 mg/kg body weight for a period of 3 consecutive days, it was found that the total duration of effect was 214 hrs. During the course of these investigations, none of the animals showed any adverse effects, in spite of being administered very heavy doses of acenocoumarin to some of the animals.

The results obtained in the present study in experimental animals are in agreement with those reported in clinical cases by W.E. Keill, (1957) and Norwich, (1959). From these results it is obvious that acenocoumarin is about 60 times more potent than ethylbiscoumacetate; the onset of action is
not a single animal showed any adverse symptoms; the onset of action is however, delayed but the total duration of action is much longer as compared to ethylbiscoumacetate. The mechanism of action seems to be mainly depression of prothrombin. As evident by the present experiments, on the whole, the drug seems to be much safer than the other existing drugs of the same class on account of low dosage and absence of toxic symptoms in doses required for effective hypoprothrombinemic effect in dogs.

We thank M/s Surhid Geigy for a generous supply of Acenocoumarin and Thrombokinase tablets.

REFERENCES