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

(*Received October 16, 1963*)

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

* This formed part of the thesis submitted to Gujarat University for the degree of M.Sc. (Medical), 1963.

(Pratt 1956). In the present study, adult, healthy dogs have been used as experimental animals.

Various methods have been utilised to study the anticoagulant properties of these drugs by different workers. The most popular of this method has been the one described by Quick (Montigel, 1962) for prothrombin estimation.

METHODS

Fifty, mongrel dogs of either sex weighing between 8 to 12 kg were used. The animals were divided into seven groups each consisting of 5 to 8 animals. The dogs were kept on a mixed diet consisting of bread, meat, vegetables and milk. The normal prothrombin values were estimated in all the animals before administration of the drug on an empty stomach. The drug was made into a uniform suspension in milk and water and administered intragastrically by means of a suitable stomach tube.

The first and the second group of animals were administered acenocoumarin 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 that acenocoumarin 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.

TABLE I

Showing the effect of acenocoumarin and ethylbiscoumacetate on prothrombin time, prothrombin index and coagulation valency

Drug & dose.	Time in hrs after administration of drug	Mean prothrombin time in sec	Standard deviation of the mean	Prothrombin Index	Coagulation valency
		Group I			
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
Ethylbiscoumacetate	0	12	± 2.121	100	100
	18	20.3	± 1.908	59.1	31
30 mg/kg body wt	42	21	± 1.458	57.1	29
	66	15	± 1.904	80	61
	90	12	± 1.225	100	100
		Group II			
Acenocoumarin	0	13	± 2.025	100	100
0.4 mg/kg	18	14	± 2.846	93.1	85
body wt	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
		Group III			
Acenocoumarin	0	14	± 1.964	100	100
	18	16	± 2.132	87.5	72
0.8 mg/kg body wt	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

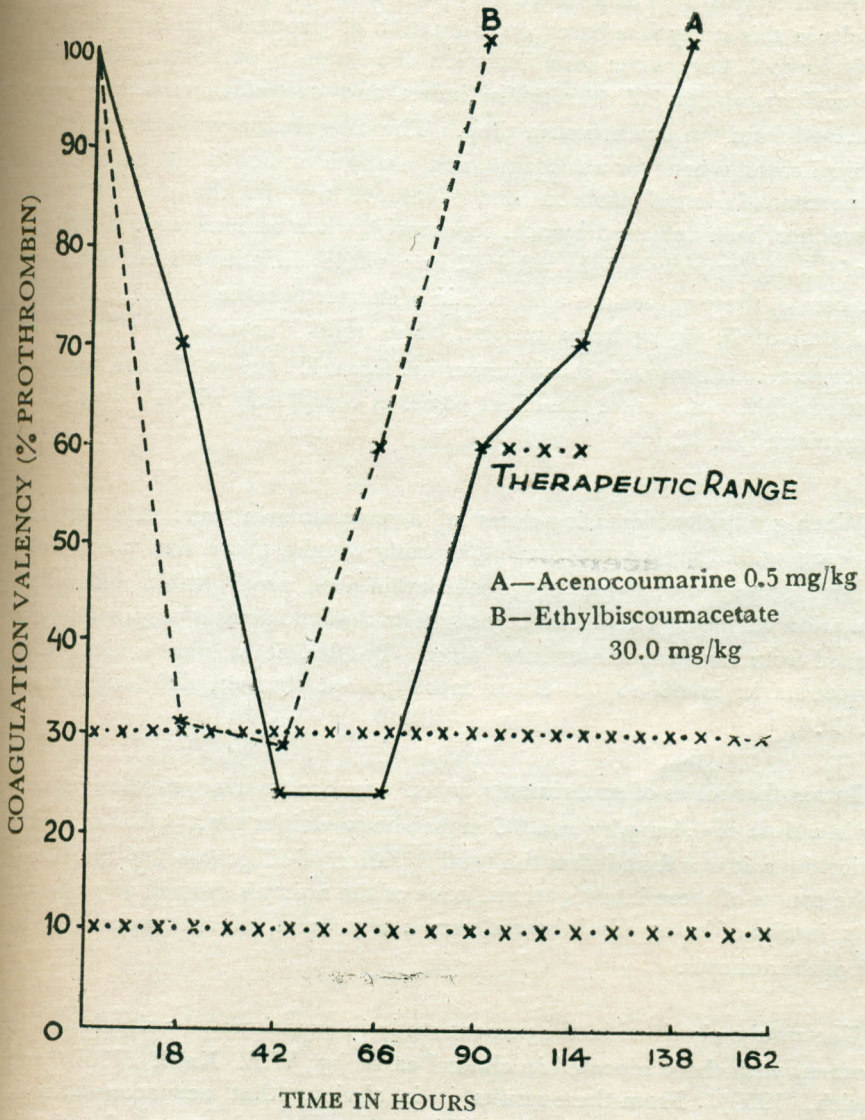
TABLE I (Contd)

Drug & ose	Time in hr. after administration of drug	Mean prothrombin time in sec	Standard deviation of the mean	Prothrombin Index.	Coagulation valency
	138	15	± 2.739	83.5	86
		Group IV			
Acenocoumarin	0	15	± 2.689	100	100
	18	18	± 2.017	83.3	66
	42	34	± 2.283	44.5	6
1.2 mg/kg	66	44	± 3.209	34.5	9
body wt	90	40	± 2.449	37.5	11
	114	33	± 2.112	45.7	17
	138	18	± 2 257	83.3	66
	162	15	± 2.738	100	100
		Group V			
Acenocoumarin	0	20	± 3.117	100	100
	18	22	± 1.873	91	80
0.4 mg/kg	42	25	± 2.989	80	60
body wt	66	60	± 4.955	33.3	8
for three	90	58	± 6.761	34.5	9
consecutive	114	51	± 1.774	39.2	12
days.	138	34	± 2.112	58 8	32
	162	26	± 1.868	75.4	55
	186	20	± 3.384	100	100
		Group VI			
Acenocoumarin.	0	17	± 5.857	100	100
	18	19	± 2.325	90	79
0·6 mg/kg	42	26	± 2.037	65	39
body wt for	66	60	± 1.779	28.3	6
three consecutive	90	60	± 3.783	28.3	6
	114	55	± 3.116	31	7.2
days.	138	47.5	± 3.865	36	10
	162	35	± 1.196	48	20
	186	25.75	± 1.149	66	40
	210	17	± 1.336	100	100

* 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.



Showing the comparative hypoprothrombinemic action of acenocoumarin and elthy biscoumacetatein in dogs after a single human equivalent dose.

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 of 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. 1).

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, inspite of being administered very heavy doses of acenocoumarin to some of the animals.

The results obtained in the persent 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

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

Keill, W.E. (1957). *Canadian Services Medical Journal*, **13**, 80.

Montigel, C. (1962). *Indian Practioner*, **15**, 805.

Neill, E.C. *et al* (1957). *Circulation*, **15**, 715.

Norwich, *et al* (1959). *South African med. J.*, **33**, 613.

Pratt, G.H. (1956). *N.Y. St. J. Med.*, **56**, 1945.

Robson, J.M. and Keele, C.A. (1956). *Recent advances in Pharmacology*, 2nd ed. p. 447, J. and Churchill Ltd., London.