Studies on Blood Coagulation Disorders in Domestic Animals Thrombelastograms of Normal Cattle and Cattle Affected with Bracken Poisoning

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Abstract. Comparative studies were made on blood coagulation in clinically healthy cattle and cattle affected with experimentally-induced bracken poisoning by recording thrombelastograph (TEG). It took 3 hours to record a TEG in normal cattle. The normal range (mean $\pm$ s.d.) of the thrombelastogram obtained from a group of 11 healthy cattle was as follows. r: 15.4 ± 2.2 , K: 4.9 ± 1.1 , r+K: 20.3 ± 2.7 , ma: 80.5 ± 2.4 and m ϵ : 420.0 ± 65.0 . The TEG of three calves fed bracken exhibited a prolongation of r, K and r+K value and a reduction of ma value in the initial stage. In addition to these findings, they showed an extraordinary pattern in which post-maximal thrombus relaxation was nearly diminished, in the hemorrhagic and the serious stage. This pattern can be explained by three conditions, thrombocytopenia, an increase of fibrinogen and an increase of heparin-like substance in blood level, occurring in bracken poisoning.

There are many animal diseases with hemorrhagic symptoms. Some of them exhibit a prolongation in blood clotting time. Studies on blood coagulation in the animal disease, however, have not been advanced. This is due to the facts that determinative techniques for the activity of blood coagulation factors are highly complex and that the determination for the aggregate function of them is difficult even if each coagulation factor is determined.

Hartert [12, 13] devised a new apparatus, thrombelastography, to catch exactly the end point of blood clotting time and the beginning of fibrinolysis by observing changes in the process of blood clotting with the lapse of time. Many studies were made on the blood disease and hemorrhagic diathesis in human beings using this apparatus in Japan [3-5, 11, 16, 22, 25, 26]. As a result,

the normal value of thrombelastogram in the human blood was determined [11, 13, 16, 23, 25]. No papers have been published, however, about the thrombelastograph (TEG) of domestic animals, except the report on the dog by Hibi [16] and that on the calf by the authors [15].

The present study was made to determine the normal range of thrombelastogram (TEGm) in cattle and to clarify changes in TEGm in experimentally-induced bovine bracken poisoning.

Materials and Methods

Principle and structure of the thrombelastography

The apparatus used in this experiment was a thrombelastography made by Hellige Company in Germany. The principle and structure of this apparatus are given in Fig. 1. It consists of five parts, cuvette, spin, mirror, light and film (kymography). The cuvette is put on a table kept constantly at

Fig. 1. Main structure of the thrombelastograph

37°C and moves by a to-and-fro system at an angle of 4°45' inside (1 period=9 seconds). The spin is dipped in the whole blood examined in the cuvette. Then a switch of the apparatus is turned on.

The resistance between the cuvette and spin begins to rise gradually as fibrin increases with progress in blood clotting. The degree of motion of the cuvette is transmitted to the spin. A reflex beam on the mirror which moves together with the spin rolling, is projected on the kymograph running at a speed of 2.0 mm per minute.

The picture projected on the kymograph is called a thrombelastograph (TEG), and the result obtained from it a thrombelastogram (TEGm). TEG exhibits a process of blood coagulation and changes in elasticity of blood fibrin.

Whole blood was collected from the jugular vein into a siliconed syringe and a test tube. By the aid of a siliconed pipette, 0.36 ml of whole blood was put into the cuvette. After the spin was dipped in it, it was covered by a liquid paraffin layer. Recording on the kymograph was begun punctually 3 minutes after blood flowed into a syringe.

The components of TEGm are given in Fig. 2. Of them, r is reaction time, which is the time in minutes required for amplitude to attain 1.0 mm after the blood flows into a syringe, K is coagulation time, which is the time in minutes required for amplitude to attain 20.0 mm after the appearance of the end point of r duration, ma is maximum amplitude in mm, and m_e is maximum elasticity of blood cake, which is given by the formula $\frac{100 \times ma}{100 - ma}$.

Test animals

Eleven clinically healthy female Holstein-Friesian calves 5–8 months of age were used for the determination of the normal value of TEGm. They were fed under the same condition throughout the

Fig. 2. Schema of thrombelastogram components

examination.

Besides, three female Holstein-Friesian calves 7 months of age were fed a calf ration containing approximately 2 kg of fresh bracken fronds per head per day. Changes in TEGm in each of the 3 stages of bovine bracken poisoning, initial, hemorrhagic and serious, as classified by the authors [29], were recorded. Another healthy Holstein-Friesian cow 7 years of age was used for the determination of the time required for the ma value to attain.

Results

TEG of clinically healthy calves

Approximately 2.5 hours were required for the ma value to develop in a healthy cow. Since the amplitude was reduced very slowly after the ma value was attained, it was possible to record TEG in cattle in 3 hours (Fig. 4).

The results of TEGm and hematological findings obtained from the whole blood of the 11 healthy calves were as follows (Table 1). r : 15.4 ± 2.2 , K : 4.9 ± 1.1 , $r+K$: 20.3 ± 2.7 , ma : 80.5 ± 2.4 and m_e : 420.0 ± 65.0 (mean $\pm$ s.d.). There was no significant correlation between the whole blood clotting time and the r value in the TEGm (Fig. 3).

TEG of calves fed bracken

Changes in TEGm and hematological findings of the calves with experimentally-induced bracken poisoning are given in Table 2.

The TEGm of 3 calves before ingestion of bracken showed the following values. r : 14.5–15.5, $r+K$: 18.0–20.0, ma : 78.0–79.0 and m_e : 354.5–376.2. These values were in approximately the same range as those obtained from a group of healthy calves (Fig. 5).

Table 1. Thrombelastograms (TEGm) and hematologic values in healthy calves

Calf No.	TEGm					Hematologic values				
	r (min)	k (min)	r+k	ma (mm)	me	RBC ($\times 10^4/\text{mm}^3$)	WBC ($\times 10^2/\text{mm}^3$)	Platelet ($\times 10^4/\text{mm}^3$)	Fibrinogen (mg/dl)	Whole blood clotting time (min)
1	15.5	5.0	20.5	82.0	455.6	891	101	60.6	617.2	10.0
2	13.5	4.0	17.5	82.5	471.4	824	142	45.8	617.2	15.0
3	15.5	3.5	19.0	84.5	545.2	1073	98	39.4	720.1	13.0
4	14.5	4.0	18.5	80.5	412.8	823	111	38.0	452.7	17.5
5	18.5	6.0	24.5	79.0	376.2	837	90	45.2	668.7	18.5
6	15.5	4.0	19.5	80.5	412.8	887	105	35.8	499.2	14.5
7	16.0	5.0	21.0	78.5	365.1	927	95	38.4	462.9	18.5
8	14.5	5.0	19.5	83.0	488.2	903	131	40.0	565.7	20.5
9	13.0	4.5	17.5	79.0	376.2	888	89	38.6	514.4	19.5
10	13.0	7.0	20.0	76.0	316.7	896	102	28.2	545.1	15.5
11	20.0	6.0	26.0	80.0	400.0	770	151	29.4	637.8	17.0
Mean	15.4	4.9	20.3	80.5	420.0	884	111	39.9	572.8	17.1
S.D.	2.2	1.1	2.7	2.4	65.0	77.9	21.3	8.7	86.7	2.4

Table 2. Thrombelastograms (TEGm) and hematologic values in calves affected with bracken poisoning

Stage	Calf No.	TEGm					Hematologic values				
		r (min)	k (min)	r+k	ma (mm)	me	RBC ($\times 10^4/\text{mm}^3$)	WBC ($\times 10^2/\text{mm}^3$)	Platelet ($\times 10^4/\text{mm}^3$)	Fibrinogen (mg/dl)	Whole blood clotting time (min)
Pre-feeding	21	15.5	4.5	20.0	79.0	376.2	951	135	60.4	799	1.95
	22	14.5	5.0	19.5	78.0	354.5	1016	103	58.0	615	16.0
	23	14.5	3.5	18.0	78.0	354.5	844	105	55.6	593	22.0
Initial	21	20.5	8.0	28.5	72.5	236.6	799	62	27.6	812	16.0
	22	22.0	11.0	33.0	71.0	244.8	993	57	13.2	664	27.5
	23	19.0	6.0	25.0	72.5	263.6	814	58	14.0	627	—
Hemorrhagic	21	219.0	∞	∞	(3.0) *	—	671	18	5.0	1332	62.0
	22	21.0	17.0	38.0	(68.0) **	—	707	38	5.4	1355	28.5
	23	15.0	8.0	23.0	70.0	233.3	772	62	10.0	632	14.0
Serious	21	312.0	∞	∞	(6.5) †	—	—	—	—	—	—
	22	21.5	18.5	40.0	64.0	117.8	—	—	—	—	—
	23	39.0	106.0	145.0	(34.5) ‡	—	831	11	3.8	1474	38.5

Remarks.

*: Value after 6 hours. **: Value after 3 hours. †: Value after 24 hours. ‡: Value after 8 hours.

In the initial stage both r and K were prolonged a little in duration and ma and me were reduced in value. In this stage a decrease was exhibited in the leukocyte and

platelet counts. The whole blood clotting time was prolonged a little in one (No. 22) of the 3 calves.

In the hemorrhagic stage calf No. 21

Fig. 3. Correlation between reaction time (r) and whole blood clotting time

showed a prolongation (219 min) in r value. The TEGm of this case exhibited only 3.0 mm in amplitude and did not attain K value or ma value even after 6 hours. In calf No. 22 both r and K value were prolonged

a little. The amplitude of this case after 3 hours was 68.0 mm and did not attain ma value.

Hematological findings of these two animals exhibited a marked decrease in leukocyte and platelet count, a high increase in blood fibrinogen and a prolongation of whole blood clotting time. Calf No. 23, however, showed no changes of TEG in the hemorrhagic stage (Fig. 6).

In the serious stage the TEG of calf No. 21 showed a marked prolongation (312 min) in r value and was only 6.5 mm in amplitude even after 24 hours. This animal did not attain K value or ma value. The TEG of calf No. 22 showed the same pattern in this stage as in the hemorrhagic stage. The TEG of calf No. 23 exhibited a marked prolongation (106.0 min) in K value. It was 34.5 mm in amplitude and no ma value was attained even after 8 hours (Fig. 7). Hematologically,

Fig. 4. Thrombelastograph (TEG) of healthy cattle

Fig. 5. Thrombelastograph (TEG) of cattle before bracken feeding

Fig. 6. Thrombelastograph (TEG) in the hemorrhagic stage of cattle affected with bracken poisoning

Fig. 7. Thrombelastograph (TEG) in the serious stage of cattle affected with bracken poisoning

this animal exhibited a marked decrease in the leukocyte and platelet count, a high decrease in blood fibrinogen level and a prolongation of blood clotting time.

Discussion

Most of the studies [1, 2, 18, 24, 27, 31, 32] on the blood coagulation in cattle were carried out on the variation of each blood coagulation factor. Little was reported concerning the variation in the aggregate function of all blood coagulation factors.

The determination used in the previous studies depended upon the macroscopic appearance of fibrin net formation and the cessation of liquefaction of blood *in vitro*. This is the reason why the results of blood coagulation obtained from bovine blood are in a wide range.

TEG devised by Hartert [12, 13] records a process of blood coagulation consecutively from the first to the fourth phase, or from the beginning of intrinsic blood coagulation to the development of fibrinolysis. This apparatus is able to screen the change of blood coagulation automatically in a short time. Thus, the normal values of TEGm in human beings reported by some workers [11, 13, 16, 23, 25, 30] are in a narrow range.

In human beings TEG is usually recorded for 2 hours [13, 25]. It was found in the present experiment that TEG of a cow

should be recorded for 3 hours, because approximately 2.5 hours were necessary to attain ma and the amplitude was reduced very slowly after ma was attained.

The normal range of TEGm in cattle was determined in 11 animals. This is the first report of TEGm in normal cattle. There was no difference in r or K value between human and bovine blood. It should be noted, however, that ma value was much larger in bovine blood (80 mm) than in human blood (50 mm). It is true that ma value is correlated with the count and function of platelets and the blood level of fibrinogen [5, 12, 13, 16]. From the fact it is supposed that an increase in bovine ma value may be due to the much higher fibrinogen level in bovine blood than that in human blood [2, 19].

In TEGm the time interval between the beginning of blood flow into a syringe and that of blood clotting is given by r value and the time when the blood cake begins to contract by K value. The sum of the blood clotting time is given by r+K. The maximum amplitude exhibits the maximum elasticity of the blood clot and is expressed as m_e [12].

In this experiment with 11 healthy calves there was no direct correlation between the clotting time of the whole blood and the reaction time (r). This fact was reported

also by other workers [5, 16, 30]. The end point of blood clotting time determined by the conventional method, Lee-White or any other modified method and by the prothrombin time method depends on the time when the blood loses its liquidity, or on the time when fibrin net begins to be formed. The end point of r in TEGm, however, shows the time when fibrin net is so developed as to move the spin of TEG. Thus the end point of r duration appears a little later in the TEG method than in the conventional macroscopic method.

In the initial stage of bracken feeding were found the prolongation in r , K and $r+K$ and the reduction in ma and $m\epsilon$. It is noticeable that in addition to these changes in the initial stage, TEG in the hemorrhagic and serious stage exhibited an extraordinary pattern in which post-maximal thrombus relaxation (hemorrhagic stage in Nos. 21 and 22, and serious stage in Nos. 21 and 23) had nearly completely been diminished.

It is known that the decrease in platelet count gives the most powerful effect on the change of r , K and ma value [5, 11, 13, 14, 16, 23, 30], but that the decrease in leukocyte count does not [30]. It is suggested that the change of r , K and ma value in each stage may be due to the decrease in platelet count. In all the animal a decrease in platelet count was observed throughout this experiment.

Some authors reported [9-11] that TEGm showed a characteristic pattern when some kinds of anticoagulant were in blood. According to them, when indandione or sintrom was added to the blood, depression or diminishing occurred to post-maximal thrombus relaxation and prolongation to r and K , but ma remained normal in value. On the other hand, the TEG of the blood containing heparin exhibited the reduction

of ma value, in addition to the prolongation of r and K . Thus it presented the characteristics of both thrombocytopenia and hemophilia.

In bovine bracken poisoning, some workers found thrombocytopenia [6, 8, 20, 21], an increase in blood fibrinogen level [6, 17] and an increase in a blood heparin-like substance [6, 7]. The present authors also observed these changes in their previous experiments [28, 29]. From these facts, it is concluded that the extraordinary pattern obtained from the TEG of the calves affected with experimentally-induced bracken poisoning in the hemorrhagic and serious stage may have been exhibited due to those three factors mentioned above.

References

- [1] Bentinck-Smith, J., Roberts, S. J., and Katz, E. M. (1960). A bleeding disease of newborn cattle. *Cornell Vet.*, **50**, 15-25.
- [2] Didisheim, P., Hattori, K., and Lewis, J. H. (1959). Hematologic and coagulation studies in various animal species. *J. Lab. clin. Med.*, **53**, 866-875.
- [3] Eguchi, K. (1971). The clinical and experimental studies on the blood coagulation by thrombelastography. I. Hypercoagulable state and the activating factors of coagulability in the arterial wall in vitro. *Fukuoka Acta medica*, **62**, 852-858.
- [4] Eguchi, K. (1971). The clinical and experimental studies on the blood coagulation by thrombelastography. II. Thrombelastography in the patients with cerebrovascular disease. *Fukuoka Acta medica*, **62**, 859-863.
- [5] Eguchi, K. (1971). The clinical and experimental studies on the blood coagulation by thrombelastography. III. Thrombelastography in the patients with liver and biliary disease. *Fukuoka Acta medica*, **62**, 864-873.
- [6] Evans, I. A., and Howell, R. M. (1962). Bovine bracken poisoning. *Nature (Lond.)*, **194**, 584-585.
- [7] Evans, I. A. (1968). The radiomimetic nature of bracken toxin. *Cancer Res.*, **28**, 2252-2261.
- [8] Fox, H. J., and France, W. H. (1954). Bracken poisoning cattle. *Vet. Rec.*, **13**, 771-772.
- [9] Fukui, S. (1958). Thrombelastograph. *Saishin*

- Igaku*, **13**, 502-510.
- [10] Fukuzawa, M., and Satake, K. (1959). Studies on a hemorrhagic diathesis by thrombelastogram. *Jap. J. clin. Med.*, **17**, 2083-2094.
 - [11] Fukuzawa, M. (1962). Studies on blood coagulation by means of thrombelastography. *Acta haemat. Jap.*, **25**, 44-65.
 - [12] Hartert, H. (1948). Blutgerinnungsstudien mit der Thrombelastographie, einen neuen Untersuchungsverfahren. *Klin. Wschr.*, **26**, 577-583.
 - [13] Hartert, H. (1951). Die Thrombelastographie. Eine Methode zur physikalischen Analyse des Blutgerinnungsvorganges. *Zschr. ges. exper. Med.*, **117**, 189-203.
 - [14] Hartert, H. (1952). Klinische Blutgerinnungsstudien mit der Thrombelastographie. I. Die Thrombocytopathien. *Deut. Arch. klin. Med.*, **119**, 293-311.
 - [15] Hayashi, T., Yamane, O., and Sako, S. (1974). Clinical findings of a cutaneous bleeding disease observed in synthetic milk feeding calves. In Proc. 78th Meeting, Japanese Society of Veterinary Science.
 - [16] Hibi, H. (1958). Studies on thrombelastography, with special reference to thromboembolism. *Acta haemat. Jap.*, **21**, 795-810.
 - [17] Howell, R. M., and Evans, I. A. (1969). Chromatographic characteristics of fibrinogen and seromucoid in bovine bracken poisoning. *J. comp. Path.*, **77**, 117-128.
 - [18] Kociba, G. J., Ratnoff, O. D., Loel, W. F., Wall, R. L., and Heider, L. E. (1969). Bovine plasma thromboplastin antecedent (Factor XI) deficiency. *J. Lab. clin. Med.*, **74**, 37-41.
 - [19] McSherry, B. J., Horney, F. D., and de Groot, J. J. (1970). Plasma fibrinogen levels in normal and sick cows. *Can. J. comp. Med.*, **34**, 191-197.
 - [20] Miura, S., and Ohshima, K. (1961). A pathological study on the first cases of bracken poisoning in cows in Japan. *Jap. J. vet. Sci.*, **23**, 347-352.
 - [21] Naftalin, J. M., and Cushnie, G. H. (1965). Hematology of experimental bracken poisoning of cattle. *J. comp. Path.*, **66**, 354-371.
 - [22] Nishi, A. (1971). Observation of plasmin on platelet by means of thrombelastograph. *J. Tokyo med. College*, **29**, 1-9.
 - [23] Oba, Y. (1959). Study on the blood coagulation by thrombelastograph. *Med. J. Osaka Univ.*, **11**, 4957-4967.
 - [24] Osbaldiston, G. W., Stowe, E. C., and Griffith, P. R. (1970). Blood coagulation. Comparative studies in dog, cats, horses and cattle. *Brit. vet. J.*, **126**, 512-521.
 - [25] Tsubokura, T. (1968). Diagnosis of hemorrhagic diathesis due to thrombocytopenia and abnormal function of platelets. *J. Hiroshima med. Assoc.*, **21**, 859-864.
 - [26] Tsubokura, T. (1971). Analysis of various abnormal blood coagulation patterns by means of thrombelastograph. *Jap. J. clin. Path.*, **19**, 544-553.
 - [27] Vagher, J. P., Pearson, B., Blatt, S., and Kaye, M. (1973). Biochemical and hematologic values in male Holstein-Friesian calves. *Amer. J. vet. Res.*, **34**, 273-277.
 - [28] Yamane, O., Hayashi, T., Sako, S., Kihara, T., Koyama, M., and Itagaki, K. (1975). Experimental studies on bracken poisoning of cattle. Clinical findings and blood figures. *Bull. Fac. Agr., Tottori Univ.*, **27**, 68-78.
 - [29] Yamane, O., Hayashi, T., Sako, S., Tatematsu, S., Takeda, K., and Fukushima, H. (1975). Studies on hemorrhagic diathesis of experimental bovine bracken poisoning. II. Heparin-like substance level in blood. *Jap. J. vet. Soc.*, **37**, 341-347.
 - [30] Yamazaki, S. (1961). Studies on blood clotting (especially by means thrombelastograph). *Tokyo J. med. Sci.*, **68**, 203-234.
 - [31] Yoshioka, Y., Fukushima, T., Uchino, T., and Nakamura, R. (1973). Studies on blood coagulation in cattle. I. Coagulation of normal cattle. *J. vet. Med. (Tokyo)*, No. 593, 629-635.
 - [32] Yoshioka, Y., Fukushima, T., Uchino, T., Nakamura, R., Yamaguchi, M., and Fujihara, H. (1973). Studies on blood coagulation in cattle. II. Coagulation of fattening cattle. *J. vet. Med. (Tokyo)*, No. 594, 685-689.