Acta vet. scand. 1984, *25*, 504—509.

From the Department of Clinical Chemistry, College of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden.

PLATELET AGGREGATION IN DOGS AFTER LIVE-VIRUS VACCINATION

By

B.-E. V. Jones

JONES, B.-E. V.: *Platelet aggregation in dogs after live-virus vaccination.* Acta vet. scand. 1984, *25*, 504—509. — Platelet aggregation induced by ADP was studied in dogs after vaccination with a modified live-virus distemper vaccine. A significant decrease in platelet counts was observed about 1 week after the vaccination. There were no consistent changes in the aggregability of the platelets. In 4 samples the aggregability was significantly increased compared to the pre-vaccination values. The observed changes will not cause a defective hemostasis in otherwise normal dogs.

ADP; thrombocytopenia; distemper.

Platelets are an important part of the mammalian hemostatic system and one of the main components of primary hemostasis. In conjunction with vaccination with some types of modified live-virus vaccines the number of circulating platelets has been found to decrease (*Straw* 1978, *Pineau et al.* 1980, *Jones* 1982). Although there are no reports on clinical hemorrhage in dogs having vaccination-induced thrombocytopenia, an additional impaired platelet function in such dogs could cause clinical problems. As it is common practice in many countries, including Sweden, to vaccinate dogs on admission to clinics and animal hospitals for surgery, the present study was undertaken to study platelet aggregation in dogs after a modified live-virus vaccination.

MATERIAL AND METHODS

Two groups of 4 dogs each were vaccinated with a modified live-virus distemper-hepatitis vaccine (Dohyvac DH, Duphar B.V., Amsterdam, Holland). The dogs were purebred Beagles, 4 males and 4 females, about 15 months old, belonging to the experimental colony of the college. This vaccination was the second

routine vaccination commonly performed on all dogs at this age, about 1 year after the initial vaccination of the pups. The dogs had not been given any medication for at least 1 month before the study and they were feed a commercial dry dog food twice a day.

Blood samples were taken before vaccination, to obtain baseline values for each dog, and 4 times after vaccination of each group af dogs as shown in Fig. 1.

The blood samples were taken about 20 h after the last feeding into plastic tubes containing 0.1 mol citrate (1:9) for platelet counting and aggregometry. Aggregation was performed in pla-

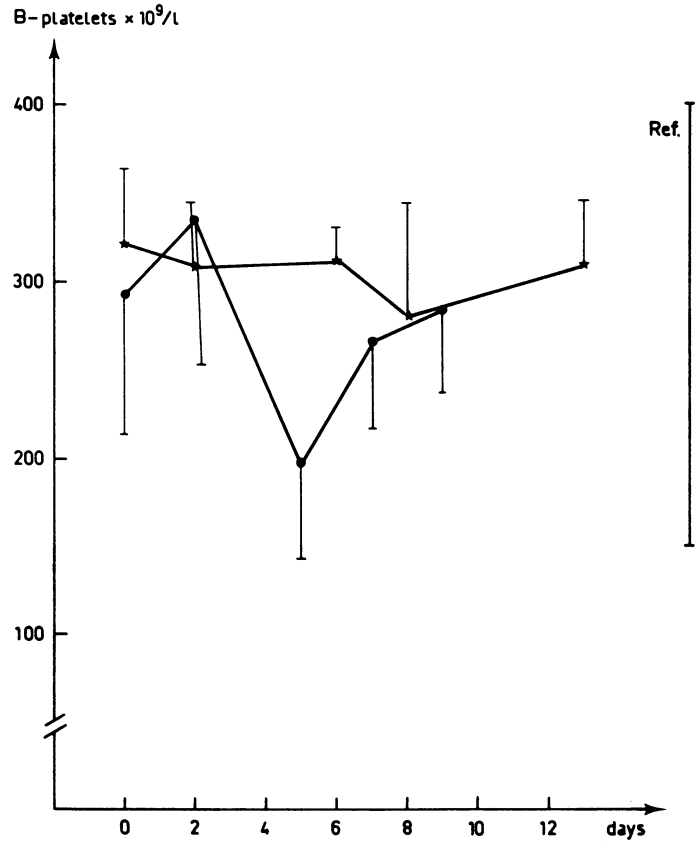

Figure 1. Platelet counts in 2 groups of dogs after vaccination with a modified live-virus vaccine. The decreased numbers seen on days 5, 7 and 8 are significantly lower than the prevaccination (0 d) value ($P < 0.05$). The reference value ($250—400 \times 10^9/l$) is shown to the right.

telet-rich plasma (PRP) with a platelet count adjusted to 200×10^9/l with platelet-poor plasma (PPP) using a Payton 300 B Aggregation Module (Payton, Searborough, Ont., Canada). The aggregating agent used was ADP (Sigma Chemicals, St. Louis, Mo., USA) in final contrations of 1.7, 8.3 and 16.6 µmol/l. The results were recorded on a chart recorder and the responses were quantitated as described by *Johnstone* (1983) using the initial velocity (A_v) and the maximum degree of aggregation (A_m). Blood samples were also obtained for determination of hemoglobin, hematocrit, total leukocyte count and differential count using EDTA as anticoagulant. The results obtained were compared in a paired t-test.

RESULTS

The number of platelets decreased in both groups of dogs 5—8 days after vaccination but remained within the reference limits (see Fig. 1). The observed platelet numbers were significantly lower than the prevaccination (0 d) value on days 5, 7 and 8. However, in one of the groups the decrease was small (8 d) although significant.

The aggregability of the platelets did not change systematically during the period studied. The aggregability was increased significantly on 4 occasions, the initial velocity (A_v) 3 times and the maximum possible aggregation (A_m) once. But they were never changed on the same day using the same concentration of ADP (see Table 1). The differences between animals were those normally seen in our laboratory.

The other blood components studied did not show any changes during this period.

DISCUSSION

Thrombocytopenia is a prominent feature of many viral diseases and there are several suggestions as to the mechanism behind this phenomenon (e.g. *Chernesky et al.* 1973, *Scott et al.* 1978).

It has been suggested that paramyxoviruses such as canine distemper virus cause agglutination and clumping of the platelets, with or without lysis of the platelets and removal of sialic acid from the platelet surface (*Pineau et al.* 1980). These effects would increase clearance of platelets from the blood stream and

Table 1. ADP-induced aggregation in dogs after modified live-virus vaccination expressed as initial velocity (A_v) and maximum degree of aggregation (A_m). Day 0 denotes the mean and range of the prevaccination value for all 8 dogs. Also day 2 is the mean for all tested dogs. Days 5—13 are means for one group of 4 dogs on each day. Mean figures followed by the same letter are significantly different ($P < 0.05$).

Final conc. of ADP	Days after vaccination			
	0	2	5	6
16.6 μmol A_v cm/min	6.3A (2.7—10.6)	6.2 (3.2—10.4)	5.2 (3.5—8.0)	7.2 (6.9—7.9)
A_m, % of max. poss.	73B (26—87)	61 (39—96)	77 (71—90)	81 (73—89)
8.3 μmol A_v cm/min	5.8C (4.3—9.4)	6.0 (3.3—13.0)	6.4 (4.8—9.2)	6.2 (5.6—6.9)
A_m, % of max. poss.	63 (26—83)	50 (26—98)	60 (53—78)	68 (55—86)
1.66 μmol A_v cm/min	4.2D (1.8—6.6)	4.3 (2.9—8.6)	4.2 (3.6—5.4)	5.7D (5.4—6.3)
A_m, % of max. poss.	29 (22—48)	31 (13—86)	20 (18—22)	39 (35—46)

Final conc. of ADP	Days after vaccination			
	7	8	9	13
16.6 μmol A_v cm/min	8.2 (7.5—9.1)	5.8 (4.2—7.9)	5.3 (3.4—7.4)	8.7A (8.0—9.2)
A_m, % of max. poss.	80 (58—94)	73 (60—95)	86B (80—90)	68 (57—85)
8.3 μmol A_v cm/min	6.6 (2.0—9.0)	5.9 (4.4—7.4)	5.3 (3.0—7.1)	7.1C (6.4—7.9)
A_m, % of max. poss.	68 (30—93)	57 (43—68)	85 (73—91)	57 (46—73)
1.66 μmol A_v cm/min	4.6 (1.5—7.8)	4.7 (3.7—6.3)	4.2 (4.0—4.4)	4.1 (2.5—5.3)
A_m, % of max. poss.	37 (17—71)	28 (15—37)	35 (33—37)	23 (11—33)

decrease the platelet life-span. This should cause a prompt decrease in platelet counts in conjunction with the viraemia seen in spontaneous disease or after vaccination, as observed by *Pineau et al.*

In a study using rabbit platelets *Scott et al.* (1978) showed that paramyxoviruses directly caused intravascular platelet ag-

gregation. They also showed that exposure of platelets to neuraminidase changed the aggregation response and caused a rapid clearance of the platelets from the circulation. The exposure of platelets to viral neuraminidase make the platelets loose surface sialic acid but this is not a likely mechanism in thrombocytopenia induced by canine distemper as the virus is reported to lack this enzyme.

In the present study and the study by *Straw* (1978) the decrease in platelet counts was at its maximum about 1 week after the vaccination. This would more likely indicate a direct effect on the platelet-forming megakaryocytes and defect in the formation process as the normal life span of the platelet is about 1 week.

In this study as well as in the earlier study by *Straw* only some animals show a substantial decrease in platelet counts. Ocassionally an animal even shows increased platelet numbers at some samplings after the vaccination. The reason for these individual differences is probably different sensitivities to the acting mechanism behind the observed thrombocytopenia.

The aggregability did not change in the present dogs in a consistent way. The significant differences observed were seen 6, 9 and 13 days after the vaccination and not with the same concentration of ADP. There is no ready explanation for these changes, but as they are all increases in aggregability they will not affect the hemostasis in a negative way. A possible explanation is that the samples showing the increased aggregability contain a large proportion of newly produced active platelets not affected by the vaccination.

Although about 25 ml of blood was drawn at each sampling there was no observable effect on the red cell parameters in this study. One of the reasons for each group being sampled every second day was to avoid such effects and a strong stimulation of the erythropoiesis as they could also influence the platelet formation and function.

The present study confirms earlier reports that the vaccination-induced decrease in platelet counts is moderate and will as such not cause hemorrhage or impaired hemostasis. The aggregation study shows that there is no consistent change in the aggregability of the platelet that will act together with the decrease in platelet numbers and give a defective primary hemostasis.

The conclusion of the present results is that vaccination will not, in otherwise normal dogs, cause a defective hemostasis. The danger of insufficient protection against e.g. distemper is most probably greater.

REFERENCES

Chernesky, M. A., R. P. B. Larke & A. G. G. Turpie: Interaction between viruses and platelets: Requirement of haemolytic activity for platelet aggregation induced by paramyxoviruses. J. gen. Virol. 1973, *21*, 205—213.

Johnstone, I. B.: Comparative effects of phenylbutazone, Naproxen and Flunixin Meglumine on equine platelet aggregation and platelet factor 3 availability in vitro. Canad. J. comp. Med. 1983, *47*, 172—179.

Jones, B.-E. V.: Platelet aggregation in dogs with decreased platelet counts caused by vaccination with a modified live-virus vaccine. Proc. 14th Nordic Vet. Congr., Copenhagen, Denmark, 6—9 July 1982, p. 422.

Pineau, S., L. W. Belbeck & S. Moore: Levamisole reduces the thrombocytopenia associated with myxovirus vaccination. Canad. vet. J. 1980, *21*, 82—84.

Scott, S., H.-J. Reimers, M. A. Chernesky, J. P. Greenberg, R. L. Kinlough-Rathbone, M. A. Packham & J. F. Mustard: Effect of viruses on platelet aggregation and platelet survival in rabbits. Blood 1978, *52*, 47—55.

Straw, B.: Decrease in platelet count after vaccination with distemper-hepatitis (DH) vaccine. Vet. Med. Small Anim. Clin. 1978, *6*, 725—726.

SAMMANFATTNING

Trombocytaggregation hos hundar efter vaccinering med levande virusvaccin.

ADP-inducerad trombocytaggregation mättes hos hundar efter vaccinering med ett modifierat levande valpsjukevaccin. En signifikant sänkning av antalet trombocyter sågs ungefär en vecka efter vaccineringen. Inga systematiska förändringar sågs av trombocyternas aggregationsförmåga. I fyra fall sågs en signifikant ökning av aggregationsförmågan jämfört med utgångsvärden före vaccinering. De observerade förändringarna efter vaccination kommer inte att ge sämre hemostas hos tidigare friska hundar.

(Received July 31, 1984).

Reprints may be requested from: B.-E. V. Jones, the Department of Clinical Chemistry, P. O. Box 7038, S-750 07 Uppsala, Sweden.