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STUDIES ON SOME ASPECTS OF PLATELET FUNCTION IN DOGS

I- Platelet Adhesiveness*

Fahri Bölükbaşı**

Köpeklerde Trombosit Fonksiyonunun Bazı Yönleri Üzerinde Araştırmalar

I- Trombosit Yapışkanlığı

Özet : Köpeklerde trombosit yapışkanlığının ölçülmesinde *in vitro* celite ve mikro cam boncuk teknikleri kullanılmıştır. Trombosit sayımları faz kontrast mikroskopla saptanmıştır.

Aspirin ve Persantin'in trombosit yapışkanlığı üzerine olan etkilerini göstermek amacıyla, ölçümler ilâcın verilmesinden önce ve sonra yapılmıştır.

Aspirin verilmeden önce beş köpekte celite tekniği ile % 50.72 ± 1.43, cam boncuk tekniği ile % 50.01 ± 1.26 olarak bulunan trombosit yapışkanlık ortalamalarının, peros aspirin verilmesinden (200 mg/Kg. beden ağırlığı) iki saat sonra alınan kanda celite metodu ile % 39.81 ± 2.81, cam metodu ile ise % 39.32 ± 2.62 değerlerine düştürkleri yani trombosit yapışkanlığının azlığı saptanmıştır ($P < 0.01$).

Ayrıca beş köpekte intravenöz Persantin enjeksiyonundan (5 mg/Kg. beden ağırlığı) 25 dakika sonra alınan kanla da trombosit yapışkanlık yüzdeleri ölçülmüştür. Persantin enjeksiyonundan önceki ve sonraki değerler, celite tekniği ile % 50.77 ± 1.09 ve % 31.58 ± 2.78 ($P < 0.001$), cam boncuk metodu ile % 50.41 ± 1.14 ve % 30.87 ± 3.87 ($P<0.01$) olarak bulunmuştur.

Sonuçlarımız gerek Aspirin ve gerekse Persantin'in kullandığımız dozlarda köpeklerde trombosit yapışkanlığını azalttıklarını, her iki metodun da kıyaslanabilir ve inanılır sonuçlar verdiklerini göstermektedir. Mamafih Persantin'in etkisinin Aspirine nazaran daha belirgin olduğu görülmüştür.

* This is partly summarized from the study, "Studies on some aspects of platelet function in dogs." which was accepted as a docentship thesis in 1971.

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Summary : The *in vitro* celite and glass beads techniques were used for measuring the platelet adhesiveness in dogs. Platelet count were made under phase contrast microscopy.

The measurements were determined before and after Aspirin or Persantin administration to reveal the effects of these drugs in platelet adhesion in this species.

By use of the celite system the platelet adhesiveness was measured before and at the second hour after Aspirin ingestion (200 mg/Kg. body weight) in five dogs and was found to be 50.72 ± 1.43 % and 39.81 ± 2.81 %, respectively ($P < 0.01$). Before and after Aspirin ingestion, the measurements determined by the glass beads method were 50.01 ± 1.26 % and 39.32 ± 2.62 %, respectively ($P < 0.01$).

Platelet adhesiveness was also measured in another group of five dogs, by taking the blood at 25th minute after the administration of Persantin intravenously as 5 mg per kilogram body weight. The values, before and after Persantin administration, were 50.77 ± 1.09 % and 31.58 ± 2.78 % by celite technique ($P < 0.001$), and 50.41 ± 1.14 % and 30.87 ± 3.87 % by glass beads method ($P < 0.01$), respectively.

These results indicate that both Aspirin and Persantin decrease the platelet adhesiveness in dogs by the doses used in this study, and both tecniques give comparable and reliable results. The effect of Persantin, however, was found to be more pronounced than that of Aspirin.

Introduction

The term "platelet adhesiveness" is generally being used to describe the property of platelets to adhere to a foreign surface[1,7] Recently there has been considerably interest in tests designed to measure platelet adhesiveness with the aims of shedding more light on mechanisms of hemostatic plug formation, and of developing empirical laboratory procedures useful in the diagnosis of certain disorders[14,15]. In the tests used to measure the platelet adhesiveness, the platelets are able to adhere to a foreign surface and to one another.

Several methods have been proposed[2,4,6,7,13,14,16,17,21,22,24,25,31,35,40,42]. The hope has been that a decrease in adhesiveness might reflect certain pathological bleeding states and an increase a tendency to thrombosis[15].

There exists the hope that certain pharmacological agents which are nontoxic and would reduce platelet adhesiveness without affecting hemostasis may eventually find clinical usefulness in the management of thromboembolic disorders. Among many others, acetyl salicylic acid and dipyridamole seem to have such an effect.

Acetyl salicylic acid (Aspirin) have been recognized to be associated with hemorrhagic episodes in man [30,38]. It is stated that Aspirin decreases platelet stickiness [5,26,34,42], and clot retraction[26]

and prolongs the bleeding time [26,38,41]. It is subsequently demonstrated that Aspirin had an effect similar to that of pyrazol compounds on platelet function, platelet survival, hemostasis and thrombus formation [11,30].

Aspirin inhibits platelet aggregation induced by collagen [30,41], antigen-antibody complex, or gamma-globulin-coated-polystyrene, connective tissue fragment [11,30,41,45] and the secondary aggregation induced by ADP [45] or epinephtrine [33] in human citrated platelet rich plasma. These indicate that Aspirin probably inhibits the release of platelet constituents. Weiss and Aledort [41] state that Aspirin inhibits the release of platelet ADP although the platelets still aggregate normally when this nucleotite is added to platelet-rich-plasma (PRP).

It has recently been observed by Doery et al.[9] that very high concentrations of Aspirin inhibit glucose uptake and lactate production by human platelets.

Hinshaw et al.[18] demonstrated that Aspirin prevents the acute shock caused by intravenous endotoxin infusion in dogs.

The long lasting effect of acetyl salicylic acid [1,33] may be related to the acetyl linkage in Aspirin [38], and to the acetylation of proteins by acetyl salicylic acid [1].

These findings may explain the increased bleeding tendency after Aspirin ingestion and suggest that the drug may have anti thrombotic properties [41] which might be a useful approach to antithrombotic therapy [43,44].

Dipyridamole (Persantin) is primarily known as a coronary vasodilator. This substance has been shown to reduce ADP-induced platelet aggregation [10,23,28,37] and adhesion of platelets to glass beads [36,39].

In vitro studies on human platelets by Emmons et al[10]. Revealed that dipyridamole, reduced ADP or epinephrine induced aggregation and increased the disaggregation rate. Mustard and Packham [30] reported that it inhibits collagen induced platelet aggregation in human and rabbit plasma, and that it also inhibits thrombin or collagen induced release of serotonin and nucleotides from rabbit platelets.

Dipyridamole decreases the rate of disappearence of exogenous adenosine from whole blood in vitro [37], and theoretically should inhibit ADP induced platelet aggregation by increasing the amount of adenosine in plasma [28,30].

Hellem[17] has found that the mean value of ADP induced platelet adhesiveness in vitro, decreased linearly with increasing concentrations of dipyridamole. Oral administration of 450 mg dipyridamole daily did not reduce the platelet adheviveness.

Rosner et al.[39] present some results obtained in vitro with this substance, which are in good aggreement with Emmon's findings [10], and state that dipyridamole antagonizes ADP.

Philp and Lemieux[36] could not demonstrate a significant effect of dipyridamole on thrombus formation in injured cortical vessels of rabbits and rats. Didisheim[8], however, found that this substance prevents thrombus formation at the injured sites of blood vessels in the rat and in arteriovenous shunts made of Teflon.

The aim of this investigation is to determine whether the celite method originally proposed by Pegrum et al.[35] and the glass beads method of Morris and Miller[25] could be used in measuring the platelet adhesiveness and whether aspirin and dipyridamole had an effect on platelet function in dogs.

Materials And Methods

All dogs tested for adhesiveness were maintained on the same practical type ration and were apparently healthy. Females were nongravid. 10 dogs were used in the experiments.

A venipuncture of the jugular vein promptly performed with a sharp 18-gauge, nonsiliconized needle (Monoject Products, Inc., U.S.A.) fitted to a polystyrene syringe. 2-3 ml of blood was drawn and discarded, avoiding problable mixture of some tissue fluid during venipuncture. A second plastic syringe of 10 ml (Burron Medical Products, Inc., U.S.A.) which contained 1 ml of 3.8 % citrate solution, was fitted to the same needle already in the vein and 9 ml of blood was drawn avoiding air bubbles. This citrated whole blood was then transfered into a silicone-treated bottle.

The measurements were performed before and after Aspirin or Persantin administration.

The blood was drawn 2 hours after Aspirin (Bayer) ingestion, 200 mg per kilogram body weight, as described for rabbits by Evans et al.[11].

Persantin (Boehringer), 5 mg per kilogram body weight, was given intravenously as used in rabbits by Philp and Lemieux[37]. The blood was taken 25 minutes after the injection of Persantin.

2 techniques have been employed in measuring the platelet adhesiveness: The glass bead method of Hardisty et al.[13] which modified by Morris and Miller[25] and the celite system of Pegrum et al.[35] which slightly modified and used in normal and bleeder swine by Cornell et al.[6]. Citrated whole blood was used in our studies to keep the experimental conditions as uniform as possible[35]. The experiments were all carried out at room temperature.

In celite technique, Celite 560 of Johns-Manville Products Corporation, NewYork, was previously washed and dried as described[6,35].

In this technique 1 ml of citrated whole blood was transfered via a polypropylen pipette, into each of two clear plastic 12x75 mm tubes of Falcon Plastics, one containing 60 mg of Celite in it. The tubes were immediately capped with Parafilm, a nonwettable product of Marathon Products. The tubes were clipped to the periphery of a wheel, 6 inches in diameter, the plate of the wheel being in a vertical position and the long axis of the tube being tangent to the rim of the wheel. It was then rotated at a constant speed of 35 rpm for 5 minutes. After removal from the wheel, the tubes were allowed to stand for 2 minutes before a sample was taken for platelet count. The time between the start of sample collection and treatment of the sample with celit never exceeded 5 minutes.

In the method described by Morris and Miller[25], 1 g of glass beads is added into one of two clear plastic tubes containing 1 ml citrated whole blood. Parafilm is used for lining the open ends of the tubes. From time of contact with the glass beads both tubes were gently inverted 10 times in 30 seconds. Immediately after mixing, the tubes were centrifuged for 10 minutes at 1000 rpm.

The citrated whole blood in celite technique and the PRP in glass bead method were rapidly withdrawn in erythrocyte counting pipets, and platelet count was performed using the method of Brecher and Cronkite[3]. The Platelet counts from the tubes containing celite or glass beads, were then substracted from the counts from the tubes without celite of glass beads. These differences were then expressed as percentages.

Results

A decrease in platelet adhesiveness was found after aspirin ingestion in all of 5 dogs (Table 1).

TABLE 1.

Adhesiveness (%) before and after Aspirin ingestion, (n=5)

		Dog number					Mean ± St. error	Level of Confidence
		1	2	3	4	5		
Celite Method	Before Aspirin	55.49	46.98	51.33	48.76	51.03	50.72±1.43	P < 0.01
	After Aspirin	50.91	37.11	38.39	35.59	37.03	39.81±2.81	
Glass Bead Method	Before Aspirin	54.45	47.53	49.32	50.90	47.85	50.01±1.26	P <0.01
	After Aspirin	48.70	39.32	39.52	33.24	35.80	39.32±2.62	

By use of the celite system, the percentages of platelet adhesiveness in 5 dogs measured before Aspirin ingestion were 55·49, 46·98, 51·33, 48·76 and 51·03 %. The average mean adhesiveness was 50·72 % with a standart error of ±1·43. These values in the same dogs after Aspirin ingestion were 50·91, 37·11, 38·39, 35·59 and 37·03 %, respectively. The average mean adhesiveness, here, was 39·81 ± 2·81 %. The difference between measurements before and after aspirin was found to be statistically significant at a level of confidence of 1 %.

By the glass bead method in the same dogs, comparable results to those of the celite technique were obtained. The individual values before Aspirin ingestion were 54·45, 47·53, 49·32, 50·90 and 47·85 % which gave an average mean adhesiveness of 50·01 ± 1. 26 %. After Aspirin ingestion, the measurements in the same dogs were 48·70, 39·32, 39·52, 33·24 and 35·80 %, respectively. The average mean adhesiveness was calculated to be 39·32 ± 2·62 %. The difference between the two averages was also found statistically significant with a probability smaller than 1 %.

Studies in another group of 5 dogs by intravenous injection of Persantin (2,6–bis (diethanolamino) – 4·8 dipiperidino pyrimido (5·4–d) pyrimidine) gave the following results (Table 2):

The values obtained before Persantin administration by use of celite system were 50·61, 53·95, 47·99, 48·96 and 52·33 % with an average mean adhesiveness of 50·77 ± 1·09. The measurements by the same method in the same dogs after Persantin treatment were 22·47, 38·28, 33,20, 35·39 and 28·55 % which gave an average mean adhesiveness of 31·58 ± 2·78 %. The probability of difference here was smaller than 0·1 %.

TABLE 2.
Adhesiveness (%) before and after Persantin Administration, (n = 5)

		Dog number					Mean ± St. error	Level of Confidence
		1	2	3	4	5		
Celite Method	Before Persantin	50.61	53.95	47.99	48.96	52.33	50.77 ± 1.09	P < 0.001
	After Persantin	22.47	38.28	33.20	35.39	28.55	31.58 ± 2.78	
Glass Bead Method	Before Persantin	53.30	48.43	47.37	52.49	50.46	50.41 ± 1.14	P < 0.01
	After Persantin	16.99	36.89	34.76	37.69	28.00	30.87 ± 3.87	

By glass bead method , the results before Persantin in the same dogs were 53.30, 48.43, 47.37, 52.49 and 50.46 %. Post Persantin measurements were 16.99, 36.89, 34.76, 37.69 and 28.00 %, respectively. The average mean values, before and after Persantin administration were 50.41 ± 1.14 % and 30.87 + 3.87 % which was calculated to be statistically different at the level of confidence of 1 %.

Discussion

The stickiness of platelets either to one another (aggregation) or to foreign surfaces (adhesiveness) seems likely a vital part in hemostasis and thrombosis. Both processes may be enhanced or inhibited by chemical substances[12,19,20,29,35,44].

It is emphasized that the factors concerned in the adhesiveness of platelets are complex and different methods in all probability measure different properties and aspects of platelets[17,32]. The glass bead method of Hardisty et al.[13] and Morris and Miller [25] and the celite system of Cornell et al.[6] probably measure the release reaction induced by contact with these surfaces[17]. It appears that these surfaces stimulate the release of ADP which is the major factor among the constituents released from the platelets in increasing adhesiveness[27].

In view of the current state of uncertainity of the numerous factors which might alter platelet stickiness, Morris and Miller[25] feel that the values obtained by use of whole blood are more valid

than those using plasma. Our results suggest that the same conclusion may be encountered in dogs when the celite and the glass bead techniques are used in measuring platelet adhesiveness.

Our preliminary trials demonstrated that mean platelet adhesiveness increases as celite or glass bead concentration increases, as expressed earlier in man and swine[6,27]. We determined that these techniques which might serve to understand the anbormal states in platelet function, could be used in dogs. Using 60 mg of celite or 1 gm of glass beads gave about a 50 % adhesiveness in normal dogs, by which the increased and the decreased adhesiveness of dog platelets would easily be reflected.

Our results indicated that both aspirin and Persantin decrease the platelet adhesiveness in dogs by the doses used in this study. The effect of Persantin, however, was found to be more pronounced than that of Aspirin (Table 3).

TABLE 3.

Mean Platelet Adhesiveness in Dogs by the Celite and Glass Beads Methods. Before and After Aspirin or Persantin Administration, (n = 10)

	Mean ± standart error	
	Celite Method	Glass Beads Method
Before Aspirin	50.72 ± 1.43	50.01 ± 1.26
After Aspirin	39.81 ± 2.81	39.32 ± 2.62
Before Persantin	50.77 ± 1.09	50.41 ± 1.14
After Persantin	31.58 ± 2.78	30.87 ± 3.87

These effects of Aspirin and Persantin may be attributed to reduction of release of platelet constituents which might alter the adhesion properties of platelets. The mechanisms of how these drugs function in decreasing the platelet adhesiveness in dogs could not be demonstrated in the present study, and still remains unknown.

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