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Decrease in platelet survival and total platelet sialic acid concentration in rats infected with *Plasmodium bergei bergei*

AUGUSTINE L. INYANG[1], DAVID OKPAKO[1] and ETIM M. ESSIEN[2*]
Departments of [1]Pharmacology and [2]Haematology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary

Suckling Wistar rats aged 3-5 weeks were infected through their dorsal tail vein with *P. berghei berghei* passed in Swiss albino mice. Platelet recovery and platelet survival using ^{51}Cr-labelled heterologous platelets obtained from adult Wistar rats were determined in the infected animals on different post-infection days and on a group of non-infected rats as controls. Total platelet sialic acid was also determined in the same groups of animals. The results showed reduced platelet recovery, shortened survival and reduced total platelet sialic acid content in the infected animals compared with control values. The reduction in total platelet sialic acid content was related to the degree of parasitaemia and reached significant levels on the 5th post-infection day. It is concluded that the shortened platelet survival and reduced total platelet sialic acid content observed in the *P. berghei* infected rats were causally related and may account for the thrombocytopaenia reported in experimental and natural malaria infections of animals and man.

Résumé

Les ratons de southe Wistor, ágés de 3 á 5 semaines. Out été infectes à partir de la veine dorsale de leur queue par une parasitose dénommée *P. berghei berghei* et transmissible à travers des souris albinos d'origin Suisse. Des plaquettes hétérogènes, obtenues des rats mûrs de souche Wister, étiquêtées ^{51}Cr et utilisées pour la guérisson et la survie des râtons out été determinées sur ces animaux à differents jours aprés l'infection et sur un groupe de rats qui ve sout pas contaminei mais employes comme moyens de contrôle. L'ensemble de l'aciole Sialique des plaquettes e été oussi déterminé dans ce même groupe d'animaux. Les resultats de celte experimentation out indiqué un ralentissement de survie et une réduction de l'acide slalique des plaquettes dans les animaux contamines par rapport aux valeurs de contrôle. Celte desuière réduction était relative au dégré du parasitisme et a atteint un uivean important le 5^{e} jour aprés l'infection. Ou est done arrivé ci la conclusion que tous les résultats constaté s ci-dessus en ce qui concerne les rats infectes par la *P. berghei* étaient en quelque sorte liés les aux centres et penvent expliques la thrombocytopénie signalei dans les infections du paludisme experimental et naturel chez les hommes et les animaux.

Introduction

Thrombocytopaenia characterises both normal and experimental plasmodium infections[1-3]. It had earlier been shown that the degree of thrombocytopaenia increased with rise in parasitaemia in *P. falciparum*-infected Aotus monkeys[4]. In *P. berghei*-infected golden hamsters or mice it has a rapid onset, and progresses to death within 5-7 days[5, 3]. However, the fall in platelet count can be reversed with chloroquine therapy, the counts attaining pre-infection levels by the 28th post-infection day[6].

The changes which mark these platelets for removal from the circulation remain largely unknown as are senescence changes in normal platelets. However, shortened platelet survival has been reported in *P. falciparum* infection in man although there was no evidence of reduced platelet production, suggesting enhanced platelet consumption as a mechanism[1].

Normal platelet senescence is associated with loss of platelet surface sialic acid or sialoglycopeptides among other changes. Since such loses have been

* Correspondence: E.M. Essien, National Institute for Medical Research, 6 Edmond Crescent (Off Murtala Mohammed Way), P.M.B. 2013, Yaba, Lagos.

associated with shortened survival, it has been suggested that they mark the cells for removal from circulation by the reticuloendothelial system[7-9]. A similar mechanism operates in some viral infections such as in Newcastle disease virus and influenza virus infections, where shortened platelet survival has also been reported[10]. Although the above view of senescence mechanism remains controversial in respect of red cells[11-13], the evidence in its favour supports its application to examine the question with respect to malaria infection. We therefore studied both platelet survival and total sialic acid content in normal and *P. berghei*-infected rats. This communication reports our findings.

Materials and methods

Animals

Twelve suckling Wistar rats aged 3-5 weeks each weighing 30-35g were used in these studies while adult Wistar rats of 300-420gm weight each, provided the platelet populations used for ^{51}Cr-labelling.

Infection

Each suckling rat was innoculated through the dorsal tail vein with about 5×10^8 parasitized red blood cells obtained from *P. berghei*-infected mice when parasitaemia reached the 12- 15% level. Suckling rats were used as adult rats overcame the infection probably because the *P. berghei* passaged in mice gave rise to strains with reduced virulence for rats although fatal in mice[14].

Parasitaemia

Parasite levels were determined by light microscopy on freshly prepared Leishman-stained blood films and results were expressed as number of parasitized red cells per 100 rbcs.

Collection of blood samples

Blood for these studies was collected from the exposed heart of adult Wistar rats anesthesized with ether and was dispensed into sterile polyprolylene vessels each containing 0.0167M acid citrate dextros (ACD), (Blood:citrate, 6:1, V/V).

Preparation of washed rat platelet suspensions

Rat platelets were washed according to the method of Ardlie[15]. Platelets for survival studies were suspended in rat platelet-poor plasma (PPP) at a final count of 1.50 - 2.50 x 10^9 platelets per litre. For sialic acid determination, the thrice washed platelets were suspended in Tris-buffered saline (TBS) containing 0.15 M NaCl, 20 mM Tris, 10 mM sodium citrate, 5.6 mM citric acid, pH 7.4(16). The platelet count was adjusted to 20 x 10^9 litre.

Platelet survival studies

These were performed in 12 suckling rats, 8 of which were *P. berghei*-infected, and 4 control (uninfected) rats. The experimental group was innoculated with the parasite 4-5 days prior to injection of ^{51}Cr-labelled rat platelets. This allowed labelling to be done just when the parasitaemia appeared, usually at a level of 1.44 ± 0.6%. The platelets were labelled with 50uCi of $Na^{51}Cr$-04b(1mCi/ml, 2.3ug/Cr/ml specific activity: Amersham, U.K.) using a modified method of Winocour[17- 18]. The modification consisted of injecting the labelled platelets into the tail vein of the rat. Two hours after the injection (the interval was allowed for complete recirculation of label) the first blood sample was taken and its parameters served as the zero hour value. Other samples were taken at 17.0, 42.5 and 66 hours after the zero hour sampling. All samples were processed at room temperature and the radioactivity counted in a Packard Autogamma Scintillation Spectrophotometer (Packard Instruments, U.S.A.). Platelet recovery was calculated by the method of Barret and Butler[19] and survival curve was plotted using the method of Ginsburg and Aster[20].

Determination of total platelet sialic acid content

A total of 40 suckling rats were used in this study (30 infected, 10 control). Washed platelet suspensions were prepared from a pool of 10 rats on each sampling day. The 30 animals were infected with *P. berghei* as described earlier[5] and their total sialic acid content was determined as parasitaemia progressed.

The washed platelets were resuspended in 1.1 ml of citrated TBS. Total platelet protein was determined in 0.1 ml volumes of lysed platelets by Lowry's method[21] with bovine serum albumin as standard.

The remaining 1.0 ml of the washed rat platelet suspension was used for determination, in duplicates, of sialic acid by the method of Aminoff [22] using

Table 1: Mean percent recovery of ^{51}Cr-labelled rat platelets in normal and *P. berghei* infected rats at sampling time.

Sampling time after infection (hrs.)	Mean & Recovery of ^{51}Cr-labelled platelets			P (Control vs Infected)
	Control (n = 4)	Infected (n = 7)	% Parasitaemia	
0	81.2 ± 19.8	66.8 ± 11.2	1.4 ± 0.6	NS (p > 0.05; t=1.58)
17.0	61.7 ± 22.2	38.8 ± 20.0	8.5 ± 4.2	NS (p > 0.05; t=1.77)
42.5	41.4 ± 15.2	7.9 ± 8.1	11.6 ± 3.0	p < 0.005; t=4.82
66.0	26.8 ± 15.7	2.77 ± 4.1	14.1 ± 2.5	p < 0.005; t=3.99

There was significant correlation between % platelet recovery in infected animals and percent parasitaemia at the 5% (r = -0.9789); t value was derived by the Student's t-Test method.
Mean values ± 1 SD are given
n = No. of animals used in the different experiments. ns = Not statistically significant.

N-acetyl neuraminic acid as standard. The results were expressed as nM sialic acid per mg of platelet protein.

Results

Platelet survival

Prepatent periods of rats infected with *P. berghei* ranged between 4-5 days. In control rats, initial recovery of injected ^{51}Cr-labelled platelets was 81.2 ± 19.8% while that in *P. berghei* infected animals was 66.8 ± 11.12. The difference was however, not statistically significant. Our recovery value for the control animals is similar to results of others[18,23]. Table 1, shows the mean recovery values as parasitaemia progressed. Mean survival time of 4.3 days and a platelet half-life of 43.3 hours were obtained for control rats. In the *P. berghei*-infected rats, the corresponding values, i.e. mean survival time of 2.01 days and platelet half-life of 21.0 hrs. were reduced by 53.3% and 51.5% respectively when compared to control values.

The mean platelet survival curve obtained is shown in Fig. 1. An inverse relationship exists between platelet survival time and percentage parasitaemia as shown by a significant negative correlation index of r = -0.98.

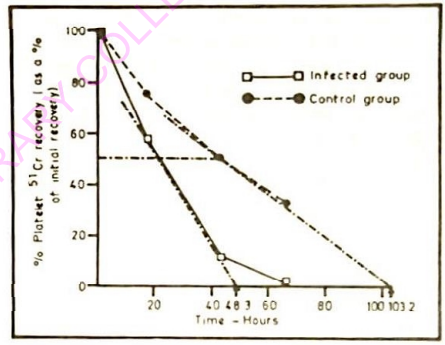

Fig. 1: Mean platelet survival curve obtained from ^{51}Cr-labelled platelets in control and *P. berghei*-infected rats.

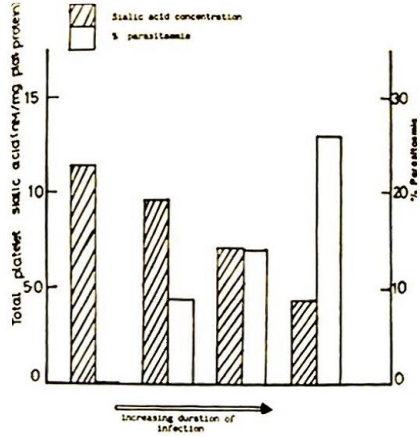

Fig. 2: Platelet sialic acid variation with rise in parasitaemia in *P. berghei* infected rats.

Table 2: Total platelet sialic acid content of normal and *P. berghei*-infected rats

Groups	No. of expts.	SA nmole/ 0.5 ml WPS	Protein mg/ 0.5ml WPS	n mole platelet SA/mg platelet protein	P value*	% Parasitaemia
Control	3	1.51	0.132	11.43 ± 1.3	NS	0.0
Infected	5	1.31	0.136	9.63 ± 2.04	NS	8.73 ± 2.04
Infected	4	0.80	0.114	7.02 ± 1.87	p = 0.005 (t = 4.19)	14.01 ± 3.0
Infected	5	0.504	0.116	4.34 ± 1.92	p = 0.005 (t = 6.63)	16.92 ± 6.77

% Parasitaemia shows mean reading from 10 animals.
Sialic acid determinations were carried out in duplicate.
* Statistical test by Student's t-test method compared total sialic acid content of control platelets with that of platelets from infected animals at different levels of parasitaemia.
Negative correlation is observed between % parasitaemia and sialic acid content of the platelets (r = - 0.9510; significant at the 5% leel).
Values represent Mean ± 1 S.D.
WPS = Washed platelet suspension; SA = Sialic acid.

Platelet sialic acid content

The mean platelet sialic acid content in the control (uninfected) rats of 11.4nM sialic acid/mg platelet protein obtained, closely approximates to the values reported by others[18]. Platelet sialic acid content reduced as parasitaemia increased (Fig. 2), showing a negative correlation index of r = - 0.95 (significant at 5% level). The time course of the survival studies when superimposed closely parallels that of the sialic acid studies suggesting some interdependence (Table 2). It was apparent from this observation that as platelet sialic acid reduced so did the percentage recovery of ^{51}Cr-labelled platelets in the infected rats fall. Both parameters became significantly different from control values on the 6th post-infection day.

Discussion

The results from our survival studies clearly showed a reduction in both mean survival time and the half-life of platelets during *P. berghei* infection in rats. A similar shortening of the survival of both labelled platelet and fibrinogen concentration has been reported in *P. falciparum* malaria infection in man[1] and these were interpreted as indicating that consumptive coagulopathy occurred during the infection. In mice, we had earlier reported haematologic changes such as depletion of fibrinogen, prolongation of PT and APTT during *P. berghei* malaria infection[3] and had also obtained results that indicate a depletion of fibrinogen levels in *P. berghei* malaria in rats (our unpublished observations). These results suggest that there was enhanced platelet/fibrinogen utilization or destruction (i.e. removal from circulation) in the disease.

In the circulation, platelet senescence is determined among other factors by age, density and changes in membrane composition including sialic acid or sialoglycopeptides[9]. For instance, the platelets whose surface sialic acid is clipped off are "marked" for removal by the reticuloendothelial system which fails to recognise the cell as self[24]. In some pathological conditions where platelet survival is compromised, it has been shown that such platelets have a lowered sialic acid content[19]. On this basis, we examined if a similar mechanism was operative during *P. berghei* infection. Our results show a progressive fall in platelet total sialic acid content as parasitaemia increased — both events had about the same time course. On this basis, we postulate that loss of sialic acid occurs from the platelet cell surface

membrane and probably also from the granules membranes but that the former accounts for subsequent platelet removal from circulation. (In this study, total platelet sialic acid concentration was used as an index of surface sialic acid content, since no study has as yet implicated granule sialic acid content with shortening of platelet survival[25-26, 10].

Platelet sialic acid can be removed by proteolytic enzymes such as trypsin, pepsin and chymotrypsin and also by neuraminidases[7,10,26]. Some studies have reported elevated levels of these proteolytic enzymes during plasmodium infection[27] although it is not known if these attain levels that can clip off surface sialic acid. Other studies have reported that during *P. berghei* infection, mouse red cell surface sialic acid is so modified that it becomes refractory to periodate oxidation[28]. Such structural alteration, if operative on platelet surface, could result in lowered platelet sialic acid concentration with resultant reduction in platelet life span as described in this study.

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