Platelet dynamics in chronic liver disease using the ^{111}In indium oxine label

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SUMMARY Platelet dynamics in chronic liver disease have been studied using ^{111}In -8-hydroxyquinolone (^{111}In -oxine) as a platelet label. In 20 patients, mainly with alcoholic cirrhosis, the platelet mean life span was reduced in 12 and the splenic platelet pool increased, often markedly so, in 15. However, the platelet count was not significantly related to mean life span, the splenic platelet pool, or spleen size. Whereas splenic platelet pool size is related to spleen size in most of the haematological 'big-spleen' syndromes, this did not apply to those patients with chronic liver disease. Technically, ^{111}In -oxine is a more appropriate platelet label than ^{51}Cr in ill and thrombocytopenic patients with liver disease.

Earlier studies of platelet dynamics in chronic liver disease have shown the frequently associated thrombocytopenia to be due to a combination of impaired thrombopoiesis, splenic pooling, and reduced platelet mean life span. Most of this work has used ^{51}Cr -sodium chromate as the platelet label, this being the isotope recommended by the International Committee for Standardization in Haematology (ICSH) for studies in man.¹ Unfortunately, the low labelling efficiency of ^{51}Cr limits the amount of radioactivity that can be administered in man,² there is evidence that ^{51}Cr is toxic to platelets,³ and there may be mechanical damage to platelets during their harvesting from relatively large volumes of blood.¹ Results of platelet dynamic studies using the ^{51}Cr label must, therefore, be interpreted with some caution, as ^{51}Cr tagged platelets may not be metabolically or structurally normal.

It is now agreed that ^{111}In -8-hydroxyquinolone (^{111}In -oxine) is an efficient and 'physiological' platelet label^{4,5} which, having a half life of 2.7 days, makes it ideally suited for measurements of platelet lifespan. Furthermore, the simple, rapid and easily reproducible technique of platelet labelling using less than 30 ml venous blood, introduced by Hawker,⁶ allows studies on ill, anaemic, and thrombocytopenic patients.

We report here our further studies of platelet dynamics in chronic liver disease now using the ^{111}In -oxine label.

Methods

PATIENTS

Twenty patients with biopsy-proven chronic liver disease were studied. All were ambulant patients who had had no recent gastrointestinal bleeding. The clinical, biochemical, and haematological data at the time of the investigation are shown in Tables 1 and 2. Similar haematological and isotopic tests were carried out on six subjects who had no hepatic or haematological disease.

Indium (^{111}In -oxine) solution for platelet labelling was obtained from the Radiochemical Centre, Amersham. Platelets were labelled by the optimal method of Hawker and his colleagues using 26 ml venous blood taken by clean venepuncture with an 18 gauge needle.⁶ Approximately 200 μCi were added to the platelet suspension for labelling.

To assess the effect of the labelling procedure on the platelet aggregation studies were performed on two samples, one taken from the initial platelet rich plasma and the other from the resuspended labelled platelet pellet immediately before reinjection. Aggregation was measured in a modified Born aggregometer⁷ using a stimulant of 1.6 μM ADP. The specimens were preincubated for 15 minutes at 37°C to remove any residual prostaglandin E1 activity present in the buffer used in this method of ^{111}In labelling.

For the platelet survival studies in patients and controls 5 ml venous blood were taken one hour after the injection of the labelled platelets and at intervals over the subsequent eight days. The one

Table 1 Clinical and biochemical data on patients

Case no.	Sex	Age (yr)	Diagnosis	Bilirubin ($\mu\text{mol/l}$)	Alkaline phosphatase (IU/l)	Aspartate aminotransferase (IU/l)	Glutamyl transpeptidase (IU/l)	Serum proteins albumin/globulin (g/l)	Prothrombin time (ratio)	Urea (mmol/l)	Creatinine ($\mu\text{mol/l}$)
1	M	65	Alcoholic cirrhosis	4	137	15	65	38/44	1.14	3.2	90
2	M	66	Alcoholic cirrhosis	15	296	10	81	35/28	1.21	12.5	195
3	M	71	Alcoholic cirrhosis	18	257	14	22	33/28	1.23	6.9	95
4	F	67	Active chronic hepatitis	35	223	129	31	34/38	1.33	3.7	77
5	F	73	Active chronic hepatitis	201	534	232	127	27/60	1.47	2.8	55
6	F	66	Alcoholic cirrhosis	52	180	14	73	45/32	1.21	2.7	70
7	M	67	Alcoholic cirrhosis	30	247	17	—	35/40	1.36	9.9	140
8	F	56	Alcoholic cirrhosis	30	255	18	42	43/35	1.15	2.2	91
9	M	86	Alcoholic cirrhosis	18	910	19	84	22/33	1.00	6.0	80
10	F	43	Alcoholic cirrhosis	39	587	13	111	35/36	1.67	4.2	70
11	M	24	Cryptogenic cirrhosis, portocaval shunt	27	136	11	15	24/29	1.67	1.6	65
12	F	49	Alcoholic cirrhosis	29	119	57	524	45/30	1.00	4.6	85
13	M	57	Alcoholic cirrhosis	23	327	43	1767	30/36	1.0	2.3	80
14	F	45	Alcoholic cirrhosis	53	327	14	167	40/50	1.13	4.5	85
15	M	68	Alcoholic cirrhosis	30	183	18	21	44/29	1.40	4.2	70
16	F	53	Alcoholic cirrhosis	18	99	15	92	39/38	1.14	3.2	—
17	F	69	Primary biliary cirrhosis	10	845	20	319	34/28	0.9	4.1	55
18	M	55	Alcoholic cirrhosis	24	243	38	860	33/37	1.07	6.0	—
19	M	51	Alcoholic cirrhosis	124	222	53	50	19/47	1.45	3.6	90
20	M	43	Cirrhosis, sarcoidosis	28	179	13	40	45/37	1.00	5.9	90

Table 2 Haematological and isotopic data on patients

Case no.	Hb (g/dl)	$\times 10^9/\text{l}$	Platelets $\times 10^9/\text{l}$	Platelet mean life span (days) (weighted mean)	Platelet survival (days)		Recovery (%)	Spleen size (g)
					Linear estimate	Log. estimate		
1	9.5	6.9	247	8.80	8.83	5.89	90	158
2	10.7	4.5	77	5.49	5.72	2.70	41	570*
3	12.4	4.2	60	9.36	9.39	4.82	20	332
4	13.1	4.0	37	11.98	11.98	7.69	20	893
5	12.1	8.4	200	9.06	9.08	4.00	30	183
6	15.9	5.1	99	7.61	7.62	3.11	11	238
7	9.3	4.8	150	7.40	7.41	3.98	70	176
8	12.2	7.0	160	7.22	7.22	4.35	38	324
9	9.0	8.5	138	5.91	5.99	3.55	53	NM
10	9.2	7.1	324	8.74	8.76	4.57	22	118
11	12.3	2.4	20	8.73	8.74	4.58	9	529
12	15.4	6.3	193	7.29	7.80	3.70	18	352
13	11.9	7.1	118	9.68	9.70	6.23	4	NM
14	15.6	9.6	77	7.06	7.10	3.14	16	193
15	16.0	7.1	127	9.40	9.41	5.51	9	310
16	13.5	5.1	72	8.17	8.17	2.58	26	260
17	11.9	6.3	175	8.36	8.55	3.78	38	NM
18	9.7	7.3	296	7.81	7.83	2.60	—	100
19	10.0	8.6	132	6.37	6.41	4.02	56	188
20	15.1	5.3	157	7.83	7.83	4.29	64	609

* At necropsy. NM = not measured.

hour sample was regarded as the 100% sample and platelet survival curves were derived from measurements of radioactivity in serial whole blood samples. The mean life span was calculated by method 1 recommended by the ICSH.¹ Both linear and logarithmic estimates of mean platelet survival were calculated and the weighted mean of these estimates was obtained and taken to be the mean platelet survival (days).

In health approximately 30–40% of the total platelet mass is in the spleen where it exists as a slowly exchanging pool. Thus, when labelled platelets are injected 30–40% of the injected platelet bound activity enters the splenic platelet pool leaving 60–70% recoverable in the peripheral blood. The platelet recovery, therefore, bears an inverse relationship to the splenic platelet pool. Platelet recovery was calculated according to the formula:

$$\frac{\text{Platelet bound activity per ml of blood in 1 hour sample} \times \text{total blood volume} \times 100}{\text{Injected platelet bound activity}}$$

The total blood volume was estimated from Nadler's formula.⁸ Spleen volume was assessed by $^{99\text{m}}\text{Tc}$ scintiscanning and the spleen volume was calculated according to the formula $v = 0.3\sqrt{a^3}$ where 'v' represented the volume of the spleen (ml) and 'a' the largest spleen surface area on an isotope scan.⁹

Results

The haematological and isotopic data concerning the 20 patients with chronic liver disease and the results of the platelet labelling studies are shown in Table 2.

Platelet survival was linear in the six patients without haematological or liver disease with mean life span of 9.58 ± 0.50 days ($\pm$ ISD) (range 8.58–10.08). This compared with ^{111}In platelet survivals in normal subjects in other series of 9.0 ± 0.71 days, 9.21 ± 0.42 days, and 9.00 days.^{10–12}

The platelet mean life span in the patients with chronic liver disease ranged from 5.49 to 11.98 days. Twelve of these patients had reduced platelet life spans. The survival curves were dominantly linear in type with the weighted mean values of mean life span approximating closely to the linear estimates. There was no correlation between the platelet counts and mean life span ($r = 0.14$).

There was no relationship between platelet counts and platelet recoveries ($r = -0.25$), the platelet recoveries representing an inverse relationship with the splenic platelet pool. Splenic size could only be assessed accurately in 17 patients but no relationship

was established between platelet count and spleen size ($r = -0.46$).

Discussion

In studies of platelet dynamics using an isotopic label optimal results are achieved with techniques which confer the least damage on platelets. Although the mean life span of platelets labelled with ^{51}Cr and ^{111}In are similar¹⁰ the ^{51}Cr method recommended by the International Committee for Standardization in Haematology¹ has several disadvantages. Firstly, it involves the harvesting of platelets from 200–500 ml whole blood. Venesection of such large volumes of blood from ill and often already anaemic patients with chronic liver disease is ethically unacceptable and also results in a reactive thrombocytosis which often invalidates any 'steady state' investigations. Secondly, sodium chromate may inhibit connective tissue induced aggregation *in vitro*.³ Although the amount of chromium which must be bound to platelets to inhibit aggregation is 10–100 times the amount of radioactive chromium bound to platelets for standard survival studies this does not necessarily imply that chromium labelled platelets are metabolically normal.³ Thirdly, a reproducible and linear platelet loss with time is not always found with ^{51}Cr labelled platelets.¹

^{111}In labelled platelets provide an excellent tool for studies with ill and possibly thrombocytopenic patients with chronic liver disease. Only 26 ml blood is required in the method described by Hawker and his colleagues⁶ and the system of labelling maintains platelets in a plasma-free state for only 60 s; at all other times the platelets are suspended in at least 37.5% autologous plasma. Uptake of ^{111}In is an active process and efficient labelling of platelets is only obtained when the patients have intact metabolic and aggregation functions.¹³ This contrasts with ^{51}Cr , which is capable of diffusing into functionally nonresponsive as well as responsive platelets. Although we did not use it in this study the high photon yield of gamma emitting ^{111}In permits excellent gamma camera imaging.²

The results of our present studies in chronic liver disease using ^{111}In as a label for the platelets confirms many of the previous findings in chronic liver disease using the ^{51}Cr label.^{14–17} Although the platelet counts in the present group of patients ranged from normal levels to as low as $20 \times 10^9/\text{l}$ there was no significant relationship between platelet count and mean life span. The mean life span of platelets in the present series was often normal or moderately reduced, though in two patients (cases 2 and 9) it was markedly reduced to less than six days.

As with the ^{51}Cr label in earlier work^{16 17} the platelet recoveries were low, indicating large platelet pools in spleens which were not themselves particularly enlarged. Again, no clear relationship emerged between the size of the splenic pool in liver disease and the degree of thrombocytopenia. This contrasts with the previously established direct relationship between the splenic platelet pool and the spleen size which has been apparent in the haematological 'big spleen' syndromes.¹⁸

Although the majority of patients in this present work had alcoholic cirrhosis there are no suggestions, as yet, that thrombocytopenia is more common in any particular diagnostic group of chronic liver disease.¹⁷ In particular, thrombocytopenia is not necessarily more frequently seen in patients with active chronic hepatitis in which increased autoantibody activity is well-recognised. Recent suggestions indicate that platelet associated immunoglobulins are common in both alcoholic and non-alcoholic liver disease and that these may be of prime importance in the pathogenesis of thrombocytopenia.¹⁹

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