Platelet Labeling for Determination of Lifespan

Emilian MLADEIMOV, Susanne GRANEGGER*, Stephan HANN**, Helmut S1NZINGER'**

Department of Nuclear Medicine, University of Vienna,
Working Group of Analytical Chemistry, Institute of Chemistry, University of Agricultural Sciences, Vienna,
VVilhelm Auerswald Atherosclerosis Research Group (ASF), Veinna, AUSTR1A

ABSTRACT

After various earlier attempts with different radiotracers ^{51}chromium and finally ^IndiumoKİne became the tracers of choice for the radiolabeling of human platelets and the subsequent monitoring of in vivo kinetics. Data on clinical application of platelet survival in atherosclerotic vascular disease and a variety of risk factors are presented. Furthermore, a new approach to use nonradioactive material (rubidium) as label for platelets allowing application in children and pregnants, where only the information on platelet survival is necessary, are discussed. The application of ^{111}Indium-oxine and cold rubidium is an underused reiiable methodology for the assessment of lifespan of human platelets for clinical diagnosis and treatment monitoring.

Key Words: Platelet survival, Plateiet radiolabeling,^{11}] In-oxine, Rubidium, Atherosclerosis, in vivo platelet functîon.

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INTRODUCTION

Determination of platelet lifespan is of clinical relevance to discover the mechanisms of abnornıal platelet consumption in hematological disorders and f.he most reliable in vivo methodology to assess platelet function in vivo in meni[1,2]!. Various radionuclides such us ^{14}C-serotonin, ^{3}H - ör ^{32}P-diisopropylfluorophosphate have been exanıined but abandoned later due to inconvenient physical characteristicst[3,6]'. The first label successfully used for platelet studies in men was ^{51}chromium introduced as chromic chloride and later on sodium chromate'[7]!. The advantage that not only platelet kinetic monitoring was possible but also the parallel imaging of abnormal deposition sites was a break-through İn 1976, when Mathew Thakur and coworkers introduced ^{m}Indium-oxinel[8]'. Since then, the use of ^{111}Indium-oxine and other complexes (tropolone, acety-

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lacetone, MPO, oxinesulphate) arnong others for labeling has attracted considerable interest, others did not succeedl[9,13]L However, its clinicaJ application due to the practical problerns of careful platelet İsolation, radiolabeling and maintaining the functional viability of the cells has not overcome by many laboratories, so that the real wide clinical application önce expected was not achieved so

Platelet survival easily can be assessed by measuring the disappearance rate of the (radio) labelled cells from circulation in healthy adults. Platelets show a linear disappearance on Cartesian coordinatesl[17]!. Dependent on their age they are destructed then predominantly in spleen and bone marrow. The normal survival of human platelets ranges between 180 and 220 hours. As the only öne in vivo platelet function parameter platelet survival reflects single as well as repeated injury, although being unspecific. Animal data clearly have shown that the extent of damaged vascular surface correlates negatively to the survival of the cellsf[7]!. Similarly, in animals as well as in human after implantation of synthetic grafts such a negative correlation between synthetic surface and platelet survival has been assessed. Excluding artefactual influence such as blood-withdrawal, haematoma, bleeding, abnormal consumption at nonvascular sites (which has to be excluded by imaging of normal distribution), platelet survival determination is a reliable parameter of number, size and activity of lesion sites (Figüre 1)U8-20]_ A significant reduction of survival only is due to a significant continuous damage to the celisi[21]'.

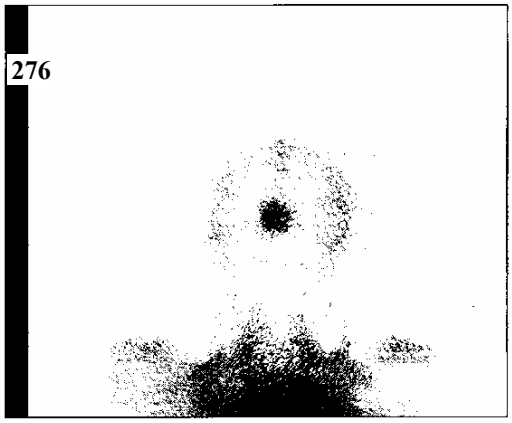

Figure 1. Image of cranial arteries 24 hours after radiolabeling of platelets with ^{111}In-oxine. Normal distribution.

Clinical Conditions

Except a variery of haematological conditions, atherosclerosis and the associated risk factors are mainly underlying an abnormal survival behaviourl[22]!.

Coronary Heart Disease (CHD)

Patients with CHD showed a most severe shortening of the in vivo life span. Among the risk factors, cigarette smoking, hyperlipidemia and diabetes are the ones showing the most significant impairment (Table l). A combination of risk factors as usually found in clinically high risk patients decreases platelet survival even further indicating impaired haemostatic balance.

Peripheral Vascular Disease (PVD)

A shortening of platelet survival in PVD is somewhat less pronounced as compared to CHD. The difference as compared to control values, however, is significant. The risk factors and again in the order of sequence smo-

Table 1. Role of risk factors (CHD)

Diagnosis	Survival (h)	n
CHD	159.8 ± 12.2	36
+ SM	137.4 ± 10.6	24
+ HY	153.7 ± 11.6	16
+ HLP	143.2 ± 10.5	17
+ SM/HY	135.3 ± 9.8	11
+ SM/HLP	129.6 ± 13.4	19
+ D	144.8 ± 15.2	21
Co	196.4 ± 12.2	16

Values in $\bar{x}$ ± SD; vs. controls are *) at p< 0.01.
SM... smoking; HY ... hypertension;
HLP ... hyperlipoproteinemia.

king, hyperlipoproteinemia and diabetes are the ones showing the most pronounced effect (Table 2).

Cerebrovascular Disease (CVD)

Shortening in platelet survival in CVD-patients is usually in the range of the ones of PVD and less pronounced as compared to CHD (Figüre 2). Interestingly, those patients showing positive lesions (+ LE) in scintigraphy the day after the labeling have platelet survival being more shortened as compared to those without lesions (- LE) (Table 3). The differences, however, are not significant (Figüre 1 shows a normal imaging of the cervical vessels).

Hyperlipoproteinemia

Platelet survival determination shows in general a good correlation to total cholesterol (r= -0.7342; p< 0.01) and LDL-cholesterol (r= -0.7584; p< 0.001) in familial hypercholesterolemia. However, no correlation to triglycerides, HDL ör VLDL-cholesterol as well as to the extent of oxidation of lipoproteins as determined via conjugated dienes ör malondialdehyde exists. in isolated hypercholesterolemia survival is usually more shortened (133 ± 11 h) vs patients with mixed hyperlipoproteinemia (145 ± 12 h), indicating that cholesterol is the key regülatör. A strong inverse relationship with J3-thromboglobulin (r= -0.7386; p< 0.001), platelet factor IV (r= -7293; p< 0.001) and even more thromboxane 62 (r- -8342; p< 0.001) exists. it has to be considered that the labeling behaviour ör human platelets and recovery are strongly inversely related to cholesterol (p< 0.01) and LDL-cholesterol (p< 0.01)![23]. Thus, in patients wlth a total cholesterol of above 300 mg/dL, longer in vitro İncubation periods are recommended in order to counterbalance the significantly irnpaired labeling efficiency.

Table 2. Platelet survival in PVD (role of risk factors)

Diagnosis	Survival (h)	n
PVD	163.7 ± 10.4	57
+ SM	144.2 ± 11.6	30
+ HY	155.8 ± 12.2	12
+ HLP	145.5 ± 9.8	26
+ SM/HY	141.6 ± 13.0	23
+ SM/HLP	132.7 ± 11.6	20
+ HY/HLP	141.8 ± 10.5	16
+ D	146.5 ± 8.9	19
+ D/HLP	136.8 ± 10.3	21
+ D/SM	133.8 ± 12.1	15
Co	196.4 ± 12.2	16

Values in $\bar{x}$ ± SD; all values are vs. controls at p< 0.01.

Drug Monitoring

Drug monitoring became öne of the most fascinating clinical applications of platelet survival studies. Application of prostacyclin and also PGE_1 teaches us that treatment inımediately reduces vascular thrombogenicity and improves survival which persists prolonged for weeks and even months, indicating a long-lasting improvernent in platelet vessel wall interaction.

While various dietary measures have been shown to be associated with a varying extent of improvernent in platelet survival rarely achieving the level of significance, treatment wlth lipid lowering agents, however has been shown to prolong significantly platelet survival due to its lipid lowering and in particular cholesterol lowering capacity (Table 4). Comparing studies whether the extent of lipid lowering either by the different drugs ör doses correlate to the extent of improvernent in systemic haernostasis, however, have not been performed yet.

Abnormal trapping of human platelets at vascular sites reflects a prolonged residence time and usually is due to an active atherosclerotic lesion site![24]. External detection have been successfully used for continuous monitoring of deposition kinetics (Figures 2a and 2b). Extent of platelet deposition was highly correlated to activity of the disease as well as shortening in platelet survival. If ima-

Table 3. Platelet survival and positive lesion imaging

Co	- LE	+ LE	
196.4 ± 12.2	166.4 ± 13.3	157.9 ± 15.2	PVD
-	161.3 ± 10.6	152.7 ± 16.3	CHD
-	165.5 ± 12.7	153.8 ± 14.1	CVD

Values in $\bar{x}$ ± SD; n= 16 each group; LE: Lesion imaged under gamma-camera; PVD: Peripheral vascular disease; CHD: Coronary heart disease; CVD: Cerebrovascular disease.

Table 4. Influence of atorvastatin on platelet survival

Before	After 8 weeks	Dose (mg)
159.5 ± 11.6	170.8 ± 10.3*)	10
157.3 ± 10.4	169.7 ± 12.0*)	20
154.2 ± 12.6	167.7 ± 11.6*)	40

Values (h) in $\bar{x}$ ± SD; n= 20 each group; *) p< 0.01 (vs. prevalue).

ging of vascular lesion sites is not required, however, the exposure to radioactivity -although minör- has to be considered![25,26]

Various iunction tests propose to assess platelet function after radiolabeling failed in their goal to become useful for clinical application. Ali these tests are taking at least 45-60 minutes. Önce waiting with the radiolabeled platelet population until viability testing has been finished, the test may reveal a normal finding, the stored labelled celi population, however, loosing dramatically in the meanwhile functional capacity. Time course and extent of decay in platelet function are r,xtremely varying on an interindividual base without available predictive information. Therefore, well trained staff needs to establish the performance of labeling on a routine base. Although a great variety of mathematical models exist, their impact for the rouline only shows minör differences, respective cornputer programs being widely available[27, 32]. Additives except prostaglandins E_L and 1_2 and eventually NO are of sorne help, however, the prostaglandins are quite expensivel[33,35]). These compounds are offering the advantage that they are not interfering with platelet function and subsequent in vivo kinetic behaviour after reinjection.

Methods for radiolabeling platelets are of clinical value, however, they are associated with the radioactive burden. Although proposed early, the use of rubidium may be quite promising in the future[17,36]}. Approaches also in our lab already l ör 2 decades ago working with amounts which could easily be administered to men, were limited by the problem of detection limits after blood withdrawal to assess the kinetic curve. This now seems to be overcome by rubidium {Rb). After incubation with 5 mg Rb labeling efficiency is determined by means ör radioactive counting (added Rb86) as well as by coupled plasma sector field mass spectrometry (ICP-SFMS). Labeling yield ranged at about 10% and in contrast to ^{in}ln-oxine was not temperature-dependent. Considering the detection limit of ICP-SFMS and labeling efficiency 0.5-1 mg Rb are finally injected into the patient allowing in vivo rnonitoring of kinetics. These findings indicate that ICP-SFMS rnonitoring of Rb-labeled blood cells is feasible and may even replace radiolabeling in those patients where subsequent gamma-camera imaging is not required.

Definitely, the advantage would be to assess values in pregnancy as well as in child-

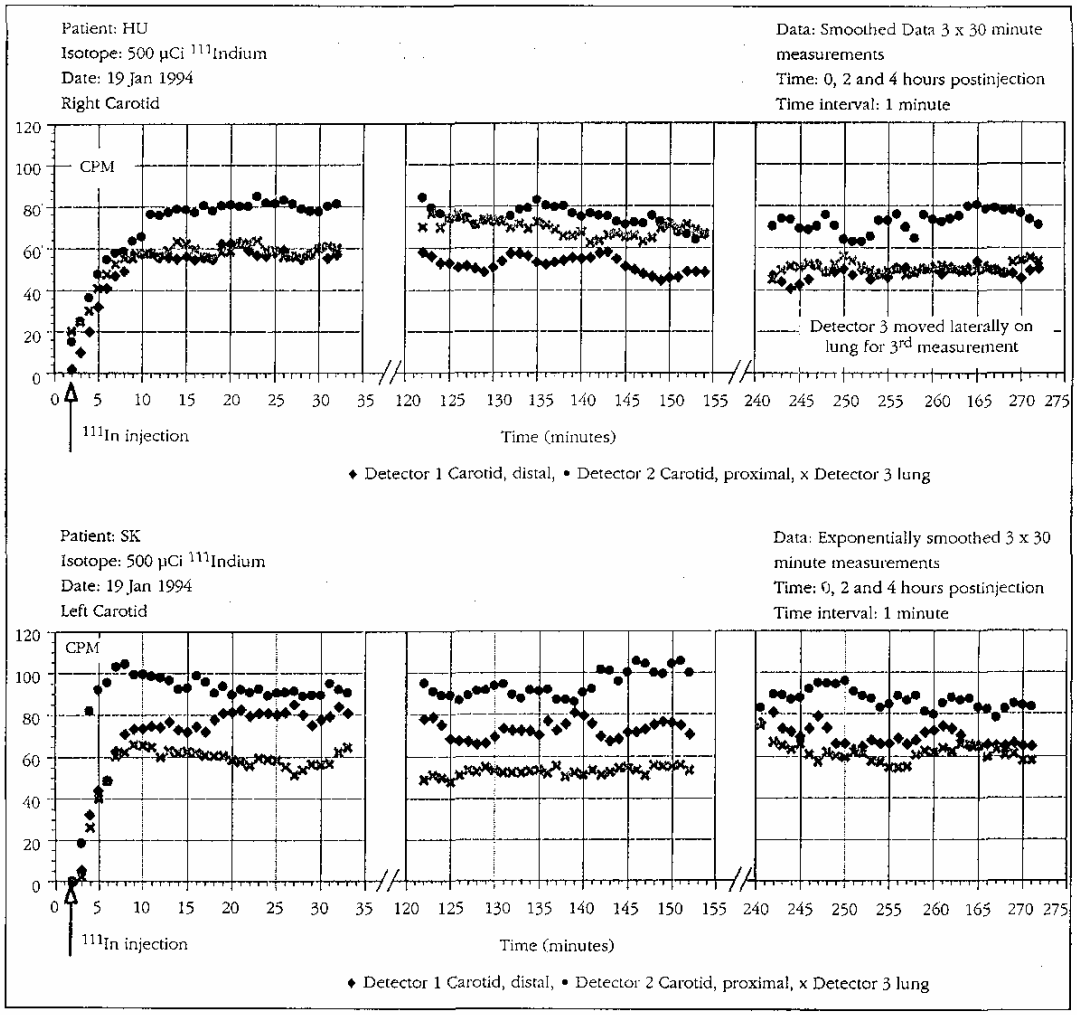

Figure 2a and 2b. Using small external detection, the kinetics over certain lesion areas can be monitored.

ren, avoiding the radioactive burden may **render** it atlractive for adults, although highly specialized **expensive** equipment is **necessary**.

External labeling with nonradioaclive compounds may yield identical artefacts vla altering the function during the extracorporal period as reported for the radioactive labels, except their additional radiation burden. Metabolic labels are requiring the administration of drugs and are furthermore only providing an indirect measure for platelet survival. Although extremely simple, determination of recovery of cyclooxygenase products (malondialdehyde, thrornboxane B_2, HHT and others) after a single high-dose acetylsalicylic acid application did not succeed either. Direct labeling may be associated with certain toxicity not allowing application in meni[37]).

There is no doubt that monitoring of platelet survival is the most valuable and reliable measure of in vivo platelet vessel wall interatcion and haemostasis. Thus, efforts to fi-

nally establish it as a routine methodology with improved cost-benefit and risk-benefit ratio are promising.

REFERENCES

1. Fuster V, Chesebro JH. Antithrombotic therapy: Role of platelet-inhibitor drugs. 1. Current concepts of throınbogenesis: Role of platelets. Mayo Clin Proc 1981;56:102-12.
2. Mustard JF, Moore S, Packham MA, Kinlough-Rathbone RL, Platelets, thrombosis and atherosclerosis. Sinzinger H, Auerswald W, Jellinek H, Feigl W (eds). Progress in Biochemical Pharmacology. Basel: Karger, 1977:312-25.
3. Heyssel RM, Silver LJ, Wasson M. The relation of blood platelets survival and distribution to 14C-serotonin distribution and excretion. Blood 1967;29: 341-53.
4. Bithell TC, Athens JW, Cartwright GE. Radioactiue diisoprophyl fluorophosphate as a platelet label: An evaiuation of İn vitro and in vivo technigues. Blood 1967;29:354-72.
5. Leeksma CHW, Cohen JA. Determination of the life of human blood platelets using labeled diisoprophylfluorophosphate. Nature 1955;157:552-3.
6. Rodrigues M, Sinzinger H. Platelet labeling-rnethodology and clinical appiications. Thromb Res 1994;76:399-432,
7. Harker LA, Slichter SJ, Sauvage LR. Platelet consurnption by arterial prosthesis: The effects of endothelialization and pharmacologic inhibition of platelet function. Ann Surg 1977;1S6:594-601.
8. Thakur ML, Welch MJ, Malech HL. Indium-111 !abelled human platelets: Studies on preparation and evaiuation of in vitro and in vivo functions. Thrornb Res 1976;9:345-57.
9. Kotze HF, Heyns A du P, Lötter MG, Pieters H, Roodt JP, Sweetlove MA, Badenhorst PN. Comparison of oxine and tropolone methods for labeling human platelets with indium-111. J Nucl Med 1991;32:62-6.
10. Vallabhajosula S, Machac J, Goldsmith SJ, Lipszyc H, Badiman L, Rand J, Fuster V. Hllndium platelet kinetics in normal human subjects: Tropolone versus oxîne methods, J Nucl Med 1986;27:l669-74.
11. Mathias CJ, Heaton WA, Welch MJ. Comparison of oxine and acetylacetone as chelates for celi labeling wîth In-Hl celi labels. J Lab Cornp Radiopharmacol 1981;18:276-82.
12. Neumann I, Strobl-Jâger E, Angelberger P, O'Grady J, Sinzinger H. A cornparison of Hlln-oxine wilh Hiln-oxine-sulphate for human platelet labeling. Thromb Haemorrh Disorders 1992;5:5-10.
13. De Vries RA, De Bruin M, Marx JJM, Van de Wiel A. Radioisotopic labels for blood celi survival studies: A review. Nucl Med Biol 1993;20:809-17.
14. Sinzinger H, Kolbe H, Strobl-Jâger E, Höfer R. A simple and safe technique for sterile autologous platelet labelling using "Monovette" vials. Eur J Nucl Med 1984;9:320-2.
15. Sinzinger H, Fitscha P, Kaliman J. Prostaglandin \2 iinproves recovery, but does not change platelet half-life and platelet uptake över active human lesion sites. Prostaglandins 1987;33:787-90.
16. Sinzinger H, Flores J, Widhalm K, Granegger S. Platelet viability (aggregation, migration, recovery) after radiolabeling of platelets from hypercholesterolemics using various tracers (oxine, oxine-sulphate, tropolone, MPO), Eur J Nucl Med 1987;14:358-61.
17. Baadenhuijsen H, De Point JJH, Daernen FJM. Uptake and efflux of RbS6 by human blood platelets. Biochim Biophys Açta 1973;298:690-701.
18. Heyns A du P, Lötter MD, Badenhorst PN, Van Reenen ÖR, Pieters H, Minnaar PC, Retief FP. Kinetics. distribution and sites of destruction of l Hindium-labelled human platelets. Br J Haematol 1980-14:269-80.
19. Sinzinger H, Virgolinİ I. Nuclear rnedicine and atherosclerosis. Eur J Nucl Med 1990;17:160-7S.
20. Wahner HW, Dunn WL, Dewanjee MK, Fuster V. Survival time and organ distribution of m-In-oKİne labeled human platelets İn normal subjects, Nucl Med Biol 1982:1:918-21.
21. Siminger H, Angelberger P, Kolbe H. Influence of incubalion time and temperature on indium-lll-oxine uptake by human platelets. Thrornb Haemost 1981;45:295.
22. Sinzinger H, Virgolinİ I, Vinazzer H. Autologous platelet labelling in thromboyctopenia. Thrornb Res 1990;60:223-30.
23. Granegger S, Flores J, VVidhalm K, Sinzinger H, Increased Iow-density lipoproteins (LDL) negatively affect human labeling. Folia Haematol 1988;115: 451-3.
24. Sinzinger H, O'Grady J, Fitscha P, Effect of prostaglandin El on deposition of autologous labelled platelets onto human atherosclerotic lesions in vivo. Postgrad Med J 1987;63:245-7.
25. Sinzinger H, Fitscha P. Monitoring of antithrornbotic activity by platelet labelling. in: O'Grady J, Linet Ol, (eds). Early Phase Drug Evaiuation in Man. London: The Macmillan Press Ltd, 1990:337-47.
26. Van Reenen ÖR, Lötter MG, Minnaar PC, Heyns A du P, Path FF, Badenhorst PN, Pieters H. Radiation döşe frorn human platelets labeled with indium-l . BrJRadiol 1980;53:790-5.
27. Baldini MG, Myers TJ. Utilitâ e limitazioni degli studi di sopra vivenza delle piastrine. Una valutazione critica. Haematologica 19SO;65:689-716.
28. Bolling DR, Murphy EA. The estimation of blood piatelet survival. VI Evaiuation of the graphical method. John Hopkins Med J 1978; 143:25-7.

29. International Committee for Standardization in Haematology. The panel on diagnostic application of radioisotopes in hernatology. Recommended methods for radioisotope platelet survival studies. Blood 1977;50:1137-44.
30. International Committee for Standardization in Haematology. Panel on diagnostic application of radionuclides. Recommended methods for indium-m platelet suruival studies. J Nucl Med 1988;29:564-6.
31. Lötter MG, Heyns A du P, Badenhorst PN, Wessels P, Martin van Zyl J, Kotze HF, Minnaar PC. Evaluation of mathematic models to assess platelet kinetics. J Nucl Med 1986;27:1192-201.
32. Snyder EL, Moroff G, Simon T, Heaton A. Members of the ad hoc platelet radiolabeling study group. Recommended methods for conducting radiolabeled platelet survival studies. Transfusion 1986;26:37-9.
33. Sinzinger H, Angelberger P, Höfer R. Platelet labelling with Hlln-oxİne: Benefit of prostacyclin (PG^) - Addition for preparation and injection. J Nucl Med 1981;22:292.
34. Radomsky MW, Palmer RMJ, Read NG, Moncada S. Isolation and \vashing of human platelets with nitric oxide. Thromb Res 1988;50:537-46.
35. Wagner O, Granegger S, Dembinska-Kiec A, Sinzinger H. Nitric oxide (NO) for radiolabeling of human platelets. in: Sinzinger H, Thakur ML (eds). Nuclear Medicine Research. Vienna: Facultas, 1989:58.
36. Del Principe D, Cesareo R, Tallarida PM, Giancarelli NG, Riggi M, Mancuso M. Labelling of blood platelets with stable tracers. Thromb Hoemost 1981;50:30-1.
37. Baker GR, Sullam PM, Levİn J. A simple, fluorescent method to internally label platelets suitable for physiological measurements. Arn J Hematol 1997;56:17-25.

Address for Correspondence:

Heimut SİNZİNGER, MD

IVilhelm Auerswald Atherosclerosis
Research Group (ASF) Vienna,
Nadlergasse l, A-1090, Vienna, AUSTRIA

e-mail: *heimut.sinzinger@akh-wien.ac.at*