The effect of elevated lipoprotein(a) on autologous platelets function: a retrospective analysis

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Abstract

Objective: Platelet labeling is used to study platelets in vivo in terms of diagnosing intravascular thrombosis, as well as studying their role and biological activity in atherosclerosis. A low labeling efficiency (LE) can negatively impair testing results. Labeling efficiency depends on various factors, including low-density-lipoprotein (LDL)-cholesterol levels in the blood. Lipoprotein(a) (Lp(a)) is a lipoprotein subclass that when elevated, is frequently associated with the premature development of cardiovascular disease through activation of different signaling pathways and cell surface receptors. **Subjects and Methods:** We retrospectively studied 51 patients with isolated elevated Lp(a) (>50 mg/dL, ranging up to 440 mg/dL) compared to patients with normal lipid profiles who underwent autologous radioactive platelet labeling during the time period of January 2001-September 2013 at the Vienna General Hospital. Platelets were radiolabeled according to ISORBE consensus. **Results:** LE was decreased in patients with elevated Lp(a). Cross-incubation of hyper-Lp(a) patients with normal Lp(a) plasma and vice versa demonstrated that platelets themselves and not the plasmatic environment are accountable for the decline in LE. Furthermore, LE positively correlated with an increase in platelet incubation time, the highest LE being seen after 30 minutes. **Conclusion:** This study determined that extremely elevated Lp(a) profiles, especially values greater than 150 mg/dL, may significantly impair platelets function such as labeling results. Platelets are responsible for the decrease in LE in hyper-Lp(a) patients. Non-HDL-Lp is the most informative parameter of impaired LE. We thus recommend to include Lp(a) in the list of parameters that need to be taken into consideration in studying autologous radiolabeled platelets.

Introduction

Platelet radiolabeling was first introduced by Thakur et al. (1976) in order to study the in in vitro and vivo and in vitro functions of platelets [1]. Radioactively labeled platelets can be used in the diagnosis of intravascular thrombosis [2-5], as well as their role and biological activity in atherosclerosis [6-8]. After being first introduced, 111-Indium-oxine has gained wide acceptance for the routine clinical use of platelet labeling [1]. The indications of platelet labeling include the determination of platelet survival, as well as the detection of active atherosclerotic lesions.

A good labeling efficiency and recovery is a key requirement for high-quality images and clinical results. Labeling efficiency of the platelets depends on several factors, such as incubation time, temperature, platelet concentration, and cholesterol levels in the blood [9-11]. In terms of cholesterol levels in the blood, studies have shown that high total and LDL- cholesterol are negatively correlated with platelet LE [12-14].

Lp(a) is lipoprotein subclass that when elevated, is frequently associated with the development of premature cardiovascular disease [15]. It contains a lipid core of LDL-cholesterol and apoB-100, incrased by apolipoprotein(a) and is synthesized and secreted by the liver [16]. Lp(a) plays a role in cardiovascular disease progression by activation of different signaling pathways and cell surface receptors, mainly alteration of platelet aggregation, decrease in fibrinolysis, recruitment of inflammatory cells and initiation of vascular remodeling [17].

Given that elevated total and LDL-cholesterol result in a decreased LE of autologous platelets, this study will investigate the role of elevated Lp(a) either isolated or in combination with elevated LDL-cholesterol in patients who underwent labeling of autologous platelets. We wish to gain knowledge on further parameters that need to be taken into consideration when labeling platelets in order to provide a high labeling efficiency in ea-

ch patient. Due to the relatively small patient population with isolated severe Lp(a) elevation, its influence on LE has not yet been studied.

Subjects and Methods

Patients

Fifty one patients with elevated Lp(a) values who underwent radiolabeling of autologous platelets between January 2001-September 2013 at the Vienna General Hospital were included in the study after chart review. Inclusion criteria consisted of available data regarding autologous radioactive platelet labeling and determination of lipid profiles, as well as Lp(a) by blood analysis. Twenty patients suffering from clinically manifested atherosclerosis with normal lipid- and lipoprotein profile served as controls. Their blood was used for cross-incubation experiments. The present study has been approved by the local institutional review board (EK-reference number: 1141/2015) and all subjects signed a written informed consent.

Procedure

Platelet radiolabeling

The consensus from the International Society of Radiolabeled Blood Elements (ISORBE) for platelet labeling was applied [18].

In each patient, approximately 16mL blood anti-coagulated with 4mL acid citrate dextrose, was drawn from a peripheral vein. The blood samples were centrifuged at 150g for 5 minutes to obtain platelet-rich plasma (PRP) [18]. The PRP was then centrifuged at 500g for 10 minutes, following which the supernatant platelet-poor plasma (PPP) was removed and stored at 37°C [18]. The remaining platelet pellet was resuspended in Tyrode's buffer to ensure a pH of 6.2 and a mean dose of 3.7MBq 111-In-oxine was added. This vial was then incubated in a water bath at 37°C for 5 minutes, followed by the addition of 5mL PPP and centrifugation of the radiolabelled platelet-pellet and buffer vial at 500g for 10 minutes [18]. The next step consisted of separating the radiolabelled platelets and determining the labeling efficiency [18]. LE was assessed as platelet bound radio-activity vs. total radioactivity. LE ranged between 10% and 94%.

Cross-incubation

Platelets of patients with isolated elevated Lp(a) vs. controls were cross-incubated in platelet free plasma at 37°C for 60 minutes. After a washing step, labeling was carried out in Tyrode-buffer at pH 6.2 for 5 minutes. Once more, LE was determined as platelet bound radioactivity vs. total radioactivity.

Platelet incubation

The correlation between LE and incubation time was also analyzed in patients with isolated elevated Lp(a). Platelets were incubated over a variety of different times beginning at five minutes and ending with 30 minutes. LE was then noted according to incubation time.

Statistical analysis

After classification of the data and analysis of normality by Kolmogorov-Smirnov tests, various parameters were analyzed using the Mann-Whitney U test. Descriptive statistics analyzed the following parameters: age, gender, labeling efficiency, cholesterol level in the blood, non-HDL-lipoprotein level in the blood, as well as lipoprotein(a) level. Values are given as p-values and odds ratio (95% confidence interval). P-values<0.05 are considered statistically significant. Analysis of data was performed using SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) v21 for Windows.

Results

Patient characteristics

In total, 51 patients, ranging in age between 36-62 years, with elevated Lp(a) values were examined. The results are seen in Table 1. For further statistical analysis, the patients were divided into isolated (normal cholesterol [i.e. <200mg/dL] and triglycerides <200mg/dL) and combined (elevated total-and LDL-cholesterol) based on Lp(a) value. The results are seen in Tables 2 and 3.

Table 1. *Patients' characteristics*

	Lp(a) 51-100	Lp(a) > 100	P
No of patients	17	34	
Gender			
Males	8	21	
Females	9	13	
Mean labeling efficiency (%)	78.0	39.2	<0.001
Mean cholesterol (mg/dL)	229.7	232.2	n.s.
Mean non-HDL-cholesterol (mg/dL)	181.0	180.1	n.s.
Mean non-HDL-Lp (mg/dL)	259.6	407.0	<0.001

n.s. not significant

Table 2. *Isolated elevated Lp(a) patients' characteristics*

	Lp(a) 51-100	Lp(a) >100	P
No of patients	8	16	
Gender			
Males	3	10	
Females	5	6	
Mean labeling efficiency (%)	90.0	45.4	<0.001
Mean cholesterol (mg/dL)	188.5	191.9	n.s.
Mean non-HDL-cholesterol (mg/dL)	139.1	145.0	n.s.
Mean non-HDL-Lp (mg/dL)	219.6	373.1	<0.001

n.s. not significant

Table 3. *Combined hypercholesterolemia and elevated Lp(a) patients' characteristics*

	Lp(a) 51-100	Lp(a) >100	P
No of patients	9	18	
Gender			
Males	5	11	
Females	4	7	
Mean labeling efficiency (%)	66.0	33.0	<0.001
Mean cholesterol (mg/dL)	270.9	254.5	n.s.
Mean non-HDL-cholesterol (mg/dL)	222.7	215.2	n.s.
Mean non-HDL-Lp (mg/dL)	299.6	440.8	<0.001

n.s. not significant

Cholesterol characteristics

Of the 51 patients, roughly half of them, 24 patients, had normal cholesterol and triglyceride levels, but isolated elevated Lp(a) values. The Lp(a) values in these 24 patients ranged from 56mg/dL to 341mg/dL. In the remaining 27 patients with combined elevated total and LDL-cholesterol and Lp(a), the lowest Lp(a) value recorded was 57mg/dL, with the highest being 386mg/dL. When analyzing the cholesterol levels, those patients with isolated elevated Lp(a) demonstrated levels ranging between 178mg/dL to 200mg/dL. Of the patients with combined hypercholesterolemia and hyper-Lp(a)emia, the cholesterol levels ranged between 217mg/dL to 327mg/dL. The association between total cholesterol and non-HDL-cholesterol with LE were not significant in patients with isolated elevated Lp(a) and in patients with hypercholesterolemia and hyper-Lp(a)emia. In terms of non-HDL-Lp, the lowest value of 196 mg/dl was found in a patient with isolated elevated Lp(a), while the highest value, 622mg/dL was recorded in a patient with hypercholesterolemia and hyper-Lp(a)emia. Interestingly, a higher non-HDL-Lp was significantly associated (P<0.001) with a lower LE, in patients with isolated elevated Lp(a) and in those with hypercholesterolemia and hyper-Lp(a)emia.

Cross-incubation experiments

In order to examine if LE depends on platelets or the plasmatic environment, cross-incubation experiments were conducted. The blood of 20 patients with normal lipid and lipoprotein profiles suffering from clinically manifested atherosclerosis was used in these experiments. Isolated platelets of the patients with isolated elevated Lp(a) were cross-incubated in the platelet-free plasma of the control samples. LE was assessed as platelet bound vs total radioactivity. The results are shown in Table 4.

Labeling efficiency

The LE of all 51 patients ranged between 10%-94%. The patient with the lowest LE of 10% had the highest Lp(a) of 386mg/dL, total cholesterol of 275mg/dL and highest non-HDL-Lp of 622mg/dL. Conversely, the patient with the highest LE exhibited a relatively low Lp(a) of 60mg/dL, total cholesterol of 188mg/dL and non-HDL-Lp value of 196mg/dL.

When the patients were divided into two groups (Lp(a) 51-100mg/dL or Lp(a)>100mg/dL), it was determined that the LE for those with an Lp(a) between 51-100mg/dL had a significantly higher LE compared to those with Lp(a) values greater than 101mg/dL (P<0.001) both in patients with isolated elevated Lp(a) and in those with hypercholesterolemia and hyper-Lp(a)emia. In order to assess the correlation between LE and incubation time, the LE of the 24 isolated elevated Lp(a) patients after various platelet incubation times were analyzed. After five minutes, the LE was 69.8±4.2. After ten minutes it increased to 70.3±5.1. Following 15 minutes, the LE was 72.6±4.2. A total of twenty minutes increased the LE to 71.8 ±3.8. After 25 minutes the LE was 73.1±4.6 and the highest LE was achieved after 30 minutes, 73.7±3.9. Therefore, the LE positively correlated with an increase in platelet incubation time with the highest LE being after 30 minutes.

Table 4. Cross-incubation platelets versus plasma

		Plasma		
		Lp(a)<30 (control)	Lp(a)>50-100	Lp(a)>100
Platelet	Lp(a)<30 (control)	91.4±1.8	90.8 ±1.7	90.4 ±2.2
	Lp(a)50-100	90.6 ±2.2	88.6 ±2.1	89.3 ±2.6
	Lp(a)>100	73.4 ±5.6	74.8 ±6.2	70.3 ±5.9

LE values in % ±SD; n=6 each subgroup
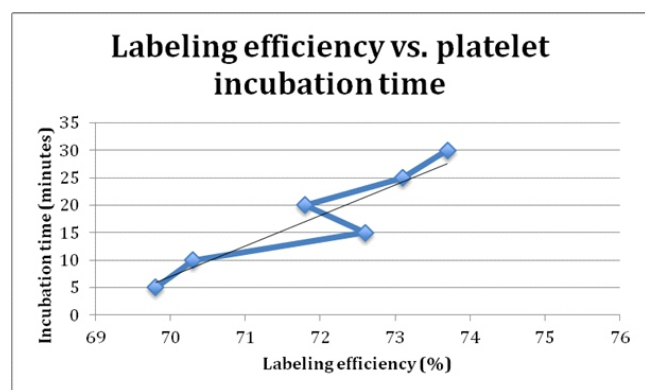
See figure 1.

Figure 1. LE vs platelet incubation time

Discussion

At present there are many methods for the diagnosis of intravascular thrombosis.

Radioactively labeled platelets in addition to their role in the diagnosis of intravascular thrombosis are used in the study of atherosclerosis. While the labeling of platelets is a routine procedure in many countries, the labeling efficiency can greatly vary. This problem is almost neglected in the literature, although it may result in a significantly impaired outcome in terms of imaging results.

In our study, the majority of patients regardless of isolated elevated Lp(a) or in combination with hypercholesterolemia, had Lp(a) values greater than 100mg/dL. An increase in Lp(a) value significantly correlated with a decrease in LE. While no other studies have examined the specific effect of Lp(a) on LE, a decrease in LE in patients with hypercholesterolemia is in accordance with other studies [12-14]. One reason for the decrease in LE could be due to the fact that hyperlipoproteinemia produces an altered platelet membrane composition [19, 20] and different fatty acid content [21], thus resulting in changed platelet membrane fluidity. Furthermore, a change in platelet kinetics, resulting in different membrane composition, can ultimately lead to the trapping of the radioactively labeled platelets in the liver and spleen [9]. Thus, patients with hypocholesterolemia, specifically hypobetalipoproteinemia, a rare genetically inherited disorder, demonstrate an increase in LE [13]. To our knowledge, this is the first study examining the relationship between LE, Lp(a) and non-HDL-Lp. We were able to show with our patient population that a high non-HDL-Lp is significantly associated with a decrease in LE. We therefore conclude that Lp(a) and non-HDL-Lp are two essential parameters that have to be known before platelet labeling in order to ensure a high LE.

While a platelet-labeling consensus has been reached, there are still some differences when it comes to incubation time. On the one hand, it seems as if 10 minutes is an appropriate incubation time to allow for appropriate radioactive binding, yet marginal platelet destruction [22]. However, a study also described the maximum LE already being reached within 60-120 seconds, hence minimizing platelet damage that can occur during prolonged incubation [10]. In this study, we demonstrated minimally increased LE in the same platelet population during prolonged incubation up to 30 minutes. Nevertheless, we believe this increase to be minimal, as we did not directly examine platelet survival after the prolonged incubation. In addition, this minimal increase might come at the expense of platelet viability. However, this was not examined in our study.

In order to examine if a lower LE is due to the platelet itself or the plasmatic environment, cross-incubation experiments were conducted in this study. Interestingly, these experiments revealed that LE depends on platelets rather than on the plasmatic environment, in accordance with other studies [13]. One theory is that hypercholesterolemia results in a change in the platelet membrane fluidity due to a change in the fatty acid composition [19, 21]. In addition, studies have shown that platelets have specific LDL-receptors, which can inhibit binding, while unrelated plasma proteins do not [23]. Furthermore, experiments have shown that the incubation of cholesterol-rich liposomes gives rise to higher cholesterol content in the platelet [24]. Interestingly, it has been demonstrated that the fatty acid content of the platelet membrane corresponds to that of the lipoprotein [25]. Therefore, it appears that the platelet itself is responsible for the uptake of lipoproteins, causing changes in its membrane, which could result in a significant and relevant decrease in LE.

One interesting point that should be discussed is under which conditions a high LE is obtained. According to Segal et al., the conditions under which optimal labeling is achieved require a surplus of cells suspended in a physiologic medium that are only exposed to a low concentration of ethanol and radiation [26]. Therefore, under normal conditions (normal cholesterol- and lipoprotein levels), a high LE for platelets is expected and obtained, since average levels of normal platelets can be found in blood. Hence, the amount of blood removed from the patients for testing should be minimal. On the other hand, when the labeling conditions are not optimal (high cholesterol, high lipoproteins), the platelets have different membrane compositions and fluidity, as well as different membrane kinetics, resulting in a lower LE, as previously discussed. Therefore, a higher amount of blood may have to be taken from these patients to ensure more radiolabeled platelets.

In conclusion, the results of our single-center study were able to demonstrate that the atherogenic lipoproteins LDL and Lp(a) most significantly impair LE and should therefore be known parameters before platelet labeling. Cross-incubation experiments demonstrated that LE depends on the platelets and not the plasmatic environment. LE positively correlated with an increase in platelet incubation time, however, this increase was only minimal.

The authors declare that they have no conflicts of interest

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