Archives of Hematology and Blood Diseases
ISSN: 2639-3581
Volume 2, Issue 1, 2019, PP: 08-11

Lp-PLA2 Activity During Iron Depletion Treatment in Primary IO Patients

Tetzlaff, Walter F[1,2,*]; Martín, Maximiliano E[1,2]; Botta, Eliana E[1,2,3]; Saez, Maria S[4]; Ferraro, Florencia M[1]; Boero, Laura E[1,2]; Sorroche, Patricia B[4]; Arbelbide, Jorge[5]; Brites, Fernando[1,2,3]; Meroño, Tomás[1,2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires, Argentina.

[2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto Fisiopatología y Bioquímica Clínica (INFIBIOC), Buenos Aires, Argentina.

[3]Consejo Nacional de Investigación Científicas y Técnicas (CONICET), Argentina.

[4]Hospital Italiano de Buenos Aires, Laboratorio Central. Buenos Aires, Argentina.

[5]Hospital Italiano de Buenos Aires, Servicio de Hematología. Buenos Aires, Argentina.

waltertetzlaff@gmail.com

***Corresponding Author:** Walter Francisco Tetzlaff, Laboratorio Lípidos y Aterosclerosis, Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.*

Abstract

***Background:** Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an inflammatory biomarker involved in atherosclerosis and cardiovascular disease (CVD). Iron stores may modify Lp-PLA_2 as higher activity levels were observed in patients with primary iron overload (IO).*

***Aim:** to evaluate the changes of Lp-PLA_2 activity and other atherosclerosis markers in patients with primary IO after iron depletion.*

***Materials and Methods:** The study initially included 20 male patients with primary IO, defined by liver histology, from which 7 were lost during follow-up and 13 completed the study (mean follow-up duration: 24±6 months). Phlebotomy treatment consisted in the removal of 1 unit of blood weekly or biweekly. We recorded traditional cardiovascular risk factors, high sensitivity C-reactive protein (hsCRP), and Lp-PLA_2 activity. Longitudinal differences were tested by paired T or Wilcoxon tests. Linear regression was used to evaluate the relationship between changes in ferritin and in Lp-PLA_2.*

***Results:** HFE mutations were present in 77% of the patients. Besides ferritin concentration (-74%), ALT (-11%) and Lp-PLA_2 activities (-14%) were reduced after iron depletion (all $p<0.05$). Linear regression showed that changes in ferritin levels explained a 60% of the variability in the changes of Lp-PLA_2 activity ($B=0.80$, $p=0.008$, $R^2= 0.60$).*

***Conclusions:** Treatment by phlebotomy significantly reduced the levels of Lp-PLA_2 activity besides its expected effects in liver markers. The implications of iron depletion for the reduction of CVD risk remain to be studied.*

Keywords: *iron overload, phlebotomy, Lp-PLA_2, hsCRP*

Introduction

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an inflammatory biomarker involved in atherosclerosis and directly associated with cardiovascular disease (CVD). It is a calcium-independent lipase that belongs to the superfamily of phospholipases A_2 (PLA_2) (1,2). It is produced mainly by inflammatory cells and circulates in the plasma linked to lipoproteins, preferentially low-density lipoprotein (LDL) (3). This enzyme has pro-inflammatory properties, since it hydrolyzes oxidized phospholipids thus releasing

lysophosphatidylcholine and oxidized free fatty acids. These compounds are known to promote vascular inflammation and endothelial dysfunction (3). Moreover, high levels of Lp-PLA_2 are present in inflamed, rupture-prone plaques, and it appears that Lp-PLA_2 is released from these plaques into the circulation (4). The activity levels of Lp-PLA_2 were associated with incident CVD and with increasing ferritin levels in an observational study. Then, a link between iron stores, oxidative stress and Lp-PLA_2 was suggested (5). In the cross-sectional analysis of our study (6), Lp-PLA_2 was found increased in patients with primary iron overload (IO) due to hereditary hemochromatosis. Moreover, Lp-PLA_2 activity was also correlated with ferritin concentration independently of body mass index (BMI), apolipoprotein B and other confounders (6). The present analysis shows the longitudinal results of the study, which aimed to evaluate the changes of Lp-PLA_2 activity and other atherosclerosis markers in patients with primary IO after iron depletion.

Materials and Methods

Subjects

The patients in this study are the ones who achieved the ferritin concentration treatment target after iron depletion treatment by phlebotomy (mean follow-up duration: 24±6 months) (6). Phlebotomy treatment consisted in the removal of 1 unit (450 to 500 mL) of blood weekly or biweekly until achieving a goal of serum ferritin between 30 and 50 µg/L. From the initial 20 male patients with primary IO, defined by liver histology, 13 completed the study. There were no significant differences in the main clinical and biochemical characteristics between these 13 and the initial 20 patients. All participants were adult males (>18 years) The present study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Review Board from Buenos Aires Italian Hospital and from the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Clinical and Biochemical Parameters

Blood samples were drawn after a 12h fast. Whole blood and serum aliquots were both stored at 4º and -70º C. Complete blood count, levels of iron, transferrin, ferritin, glucose, urea, creatinine, total bilirubin, apolipoproteins and ferritin, as well as the activities of liver enzymes were determined by automatized assays. C reactive protein was measured by a high sensitivity assay (hsCRP) (Roche Diagnostics, Mannheim, Germany). Measurement of Lp-PLA_2 activity was performed by an *in-house* radiometric assay as previously reported (6). Impaired fasting glucose was defined according to the American Diabetes Association criteria (7).

Genotyping

The *HFE* gene mutations were studied by PCR-RFLP as described previously (8). The genotypes were assigned according to the patterns observed with a UV transilluminator.

Statistical Analysis

Distribution of all variables was analyzed using the Shapiro-Wilks test. Mean ± standard deviation (SD) or median (interquartile range) were used to describe normally or skewed distributed variables, respectively. The differences in qualitative variables were assessed by McNemar test. Longitudinal differences were tested by paired T test or Wilcoxon test according to data distribution. Linear regression was employed to assess the relationship between changes in ferritin and Lp-PLA_2. Tests were considered statistically significant at $p < 0.05$. The statistical software employed were INFOSTAT (Group INFOSTAT, Córdoba, Argentina) and SPSS 19.0 (Chicago, Illinois, USA).

Results

HFE mutations were present in 77% of the patients. Out of these, four presented HFE-C282Y homozygosis, two H63D homozygosis and one C282Y/H63D double heterozygosis. At baseline, 5 of the patients were in an advanced phase of treatment (ferritin <300µg/L). Among the others, two patients showed ferritin levels >1000 µg/L. General clinical and biochemical characteristics are shown in table I.

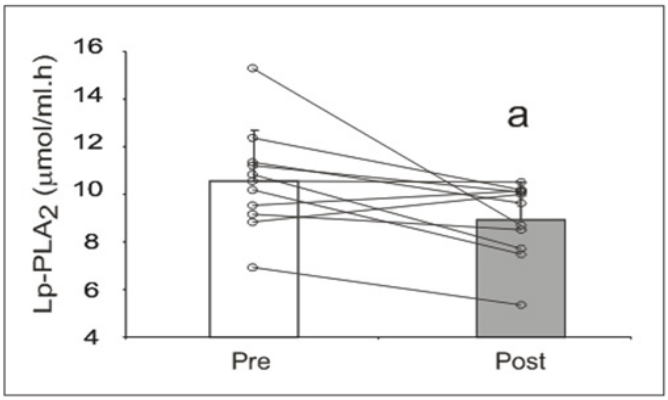
Mean age was 51 ± 12 years and mean BMI: 29 ± 4 kg/m^2. Impaired fasting glucose was present in 65% and 50%, at baseline and post-phlebotomy evaluations, respectively. However, no significant differences in glucose levels were observed during treatment. Similarly, at baseline 23% presented hsCRP > 2 mg/L, while post-phlebotomy no patient showed elevated hsCRP ($p = 0.111$).

Among liver function markers, only alanine amino transferase activity was significantly reduced by phlebotomy treatment [pre: 35 (29-45) *vs.* post: 31 (22-33) U/L; paired differences (95 CI): -10 [(-35) – (-2)] U/L, p=0.02].

Table 1. *Differences between pre and post phlebotomy treatment in patients with primary iron overload (n = 13).*

	Pre	Post	Differences (IC 95%)		P
			Inf.	Sup.	
Iron (µg/dL)	146 ± 50	151 ± 60	-40.5	26.5	0.642
TSat (%)	57 ± 24	58 ± 32	-16.4	8.8	0.508
Ferritin (µg/l)	284 (146 - 691)	74 (47 – 381)	58.6	1312.1	<0.001
Glucose (mg/dl)	97 ± 22	87 (78 – 114)	-25.9	10.5	0.754
TG (mg/dl)	142 (101 - 205)	150 ± 71	-14.6	57.4	0.220
TC (mg/dl)	178 ± 39	179 ± 27	-32.2	29.3	0.919
HDL-c (mg/dl)	42 ± 10	44 ± 9	-6.6	2.6	0.364
LDL-c (mg/dl)	116 ± 36	110 (85 – 141)	-31.9	33.8	0.952
APO A-1 (mg/dl)	136 ± 21	127 ± 11	-8.8	18.3	0.442
APO B (mg/dl)	79 ± 22	76 ± 23	-16.4	14.4	0.886
hsCRP (mg/l)	1.0 (0.5 – 2.0)	0,7 ± 0,5	-0.04	2.5	0.130

TSat, transferrin saturation; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein; hsCRP, high sensitive C reactive protein. Data are shown as mean ± SD or median (interquartile range) according to data distribution.



Lp-PLA2, lipoprotein associated-phospholipase A2.

Fig 1. *Activity of lipoprotein associated-phospholipase A2 (Lp-PLA2) in patients with IO (n = 13), both pre and post phlebotomy treatment. p <0.05.*

The changes induced by phlebotomy treatment are shown in Table I. No statistically significant differences were observed in glucose or lipid metabolism parameters. Conversely, Lp-PLA_2 activity was significantly reduced post-phlebotomy (Figure 1).

Attesting for the close relationship between iron stores and Lp-PLA_2 activity, linear regression tests showed that changes in ferritin levels during treatment explained a 60% of the variability in the changes of Lp-PLA_2 activity (B=0.80, p=0.008, R^2= 0.60).

Discussion

The present study shows that Lp-PLA_2 activity decreased following iron depletion by phlebotomy treatment in patients with primary IO. In addition, as expected ALT activity was reduced showing an impact of phlebotomy over the indicators of liver function which was not followed by changes in plasma lipids.

Tsimikas *et al.* showed that LDL-C, male gender and ferritin concentration were significant independent predictors of Lp-PLA_2 activity in 765 middle aged-adults (5). Such results agree with a role of iron in atherosclerosis through Lp-PLA_2. In line with this hypothesis, Zacharsky *et al.* performed an iron reduction trial in subjects with peripheral arterial disease, the FeAST trial (9). While no positive effect was observed in the iron reduction arm, an interesting interaction between age and iron reduction was observed. Indeed, in a sub-analysis of the trial, younger patients assigned to iron reduction showed a lower risk of CVD events than the control group (10). In addition, and as a possible explanation to the results, inflammatory markers, such as interleukin (IL)-6 and hsCRP were closely related to iron levels (11). Unfortunately, Lp-PLA_2 activity was not measured in the FeAST trial.

In our cross-sectional analysis, we observed that male patients with primary IO displayed increased Lp-PLA_2 activity, partially explained by high ferritin levels. We extend those results by showing that iron reduction was sufficient to decrease plasma Lp-PLA_2 activity. Since Lp-PLA_2 is not only a marker of coronary disease, but an important player in the development of advanced atherosclerosis plaques, the prospect of iron reduction to reduce the activity of Lp-PLA_2 and cardiovascular risk should be explored in future studies.

In conclusion, Lp-PLA_2 levels decreased with iron depletion by phlebotomy, in patients with primary IO. Such result may bring support to iron depletion as an option for the reduction of CVD risk. Future studies in patients without overt IO are necessary to confirm such association.

Acknowledgements

The authors wish to acknowledge the collaboration from the members of Buenos Aires Italian Hospital.

References

[1] Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2006 Nov; 26(11):2523-9.

[2] Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, et al. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet. 2010 May 1; 375(9725): 1536-44.

[3] Yang L, Cong HL, Wang SF, Liu T. AMP-activated protein kinase mediates the effects of lipoprotein-associated phospholipase A2 on endothelial dysfunction in atherosclerosis. Exp Ther Med. 2017 Apr;13(4):1622-9.

[4] Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. Am J Cardiol. 2008 Jun 16;101(12A):41F-50F.

[5] Tsimikas S, Willeit J, Knoflach M, Mayr M, Egger G, Notdurfter M, et al. Lipoprotein-associated phospholipase A2 activity, ferritin levels, metabolic syndrome, and 10-year cardiovascular and non-cardiovascular mortality: results from the Bruneck study. Eur Heart J. 2009 Jan; 30(1): 107-15.

[6] Merono T, Gomez L, Sorroche P, Boero L, Arbelbide J, Brites F. High risk of cardiovascular disease in iron overload patients. Eur J Clin Invest. 2011 May; 41(5): 479-86.

[7] Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes- 2018. Diabetes Care. 2018 Jan; 41(Suppl 1): S13-S27.

[8] Merono T, Brites F, Dauteuille C, Lhomme M, Menafra M, Arteaga A, et al. Metabolic alterations, HFE gene mutations and atherogenic lipoprotein modifications in patients with primary iron overload. Clin Sci (Lond). 2015 May; 128(9): 609-18.

[9] Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. JAMA. 2007 Feb 14;297(6):603-10.

[10] Zacharski LR, Shamayeva G, Chow BK. Effect of controlled reduction of body iron stores on clinical outcomes in peripheral arterial disease. Am Heart J. 2011 Nov;162(5):949-57 e1.

[11] Depalma RG, Hayes VW, Chow BK, Shamayeva G, May PE, Zacharski LR. Ferritin levels, inflammatory biomarkers, and mortality in peripheral arterial disease: a substudy of the Iron (Fe) and Atherosclerosis Study (FeAST) Trial. J Vasc Surg. 2010 Jun;51(6):1498-503.

Citation: **Tetzlaff Walter F, Martín Maximiliano E, Botta Eliana E, Saez Maria S, et. al.** *Lp-PLA2 Activity During Iron Depletion Treatment in Primary IO Patients. Archives of Hematology and Blood Diseases. 2019; 2(1): 08-11.*