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Short Communication

Platelet-derived growth factor A mRNA in platelets is associated with the degree of hepatic fibrosis in chronic hepatitis C

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Abstract:

Introduction: Transforming growth factor beta 1 (TGFB1) and platelet-derived growth factor (PDGF) are the main cytokines related to hepatic fibrogenesis. **Methods:** RNA isolated from the platelets and hepatic tissue of 43 HCV carriers was used for quantitative polymerase chain reaction to determine TGFB1, PDGFA, and PDGFB RNA expression. **Results:** The mRNA expression of PDGFA in platelets was significantly lower in the group with advanced fibrosis than in the group with early-stage fibrosis. TGFB1 was more frequently expressed in platelets than in hepatic tissue, which was different from PDGFB. **Conclusions:** A pathway mediated by overexpression of TGFB1 via PDGFA in megakaryocytes could be involved in the development of fibrosis.

Keywords: Hepatic fibrosis. PDGF. TGFB.

Hepatic fibrosis, a consequence of various chronic stimuli, including infection with hepatitis C virus (HCV), is characterized by the progressive accumulation of extracellular matrix (ECM) components in the hepatic parenchyma, which leads to a progressive replacement of the functional tissue with scar tissue[1].

Hepatic stellate cells (HSCs) are considered the main cells producing ECM proteins[2] and their activation and proliferation are mediated by two cytokines, transforming growth factor beta 1 (TGFB1) and platelet-derived growth factor (PDGF). Both are secreted by HSCs, platelets, and other cells[3,4].

Transforming growth factor beta 1 is the major profibrogenic cytokine in the liver because of its contribution to fibrosis development via HSC activation and the regulation of gene expression associated with signaling cascades related to fibrosis[5,6].

Platelet-derived growth factor is the most potent mitogen for HSCs[7]. There are four different PDGF isoforms[8] and the PDGFB isoform is considered the most important mitogen for HSCs[7-9]. However, studies have demonstrated that PDGFA can also be considered an important profibrogenic agent, acting via TGFB1 induction[10].

Considering that platelets are major sources of TGFB1 and PDGF, which are growth factors involved in the development of liver fibrosis associated with chronic hepatitis C, and that studies have already demonstrated that platelets that accumulate in the liver during chronic hepatitis may be involved in hepatic fibrosis[11], the aim of this study was to evaluate the expressions of PDGFA, PDGFB, and TGFB1 in hepatic tissue and platelets from HCV-infected patients presenting with varying degrees of hepatic fibrosis.

Aliquots of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral venous blood and fragments of hepatic tissue were collected from 43 HCV-infected patients who presented at the Department of Internal Medicine, Gastroenterology Division, Botucatu Medical School, São Paulo State University [*Universidade Estadual Paulista* (UNESP)], Botucatu, SP, Brazil. The inclusion criteria were naïve patients infected with HCV genotype 1 who were aged >18 years, had undergone a liver biopsy before the start of antiviral treatment, and had signed informed consent. The exclusion criteria were hepatitis B virus- or human immunodeficiency virus-positive

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serology, antiviral treatment before liver biopsy, the presence of other hepatic diseases, and pregnant women. The 43 patients included in this study were representative of patients seen in the service globally who have the characteristics of the inclusion criteria.

The patients were divided into two groups according to the degree of fibrosis determined in liver biopsies. The fragments were analyzed by a pathologist who used the METAVIR score system to evaluate fibrosis progression[12]. The patients were assigned to group 1 (G1) (patients with lower stages of fibrosis - F1 or F2) or group 2 (G2) (patients with higher degrees of fibrosis - F3 or F4). Information about sex, age, HCV genotype, fibrosis degree, and alcohol abuse (defined as >40g per day for women and >80g per day for men) was obtained from the patient's medical registers.

Whole-blood samples were aliquoted and plasma was isolated after centrifugation at 1,312 × *g* for 3 min. Platelet pellets were obtained according to Padovani et al.[13].

Hepatic tissue samples were collected using RNAlater® (Thermo Fisher Scientific, Inc., CA, USA) and stored at -70ºC until ribonucleic acid (RNA) extraction. RNA from hepatic tissue was isolated using the spin (SV) Total RNA Isolation System kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's specifications.

Ribonucleic acid from platelets was obtained according to Padovani et al.[13]. RNA extracted from hepatic tissue and platelets was used as a source to perform reverse-strand complementary deoxyribonucleic acid (cDNA) synthesis using the High-Capacity cDNA Archive kit (Thermo Fisher Scientific, Inc., CA, USA), according to the manufacturer's specifications.

TaqMan Universal polymerase chain reaction (PCR) Master Mix (Thermo Fisher Scientific, Inc., CA, USA) was used to evaluate the relative mRNA expression levels of PDGFA, PDGFB, and TGFB1 in the hepatic tissue and platelets. Primers and probes for the target genes (PDGFA: Hs00964426_m1; PDGFB: Hs00966522_m1; TGFB1: Hs00998133_m1) and the reference gene (ACTB: Hs01060665_g1) were obtained commercially (TaqMan® Gene Expression Assays; Thermo Fisher Scientific, Inc., CA, USA). An endogenous reference gene (β-actin) was used to normalize the expression levels of target genes All measurements were performed in duplicate.

Relative quantification of RNA in hepatic tissue and platelets from each sample was performed using a pool of RNA as the control; this was denominated the F1 pool, which was extracted from hepatic tissue obtained from treatment-naïve patients with HCV genotype 1 and mild fibrosis (F1) (the available group closest to fibrosis absence).

Relative RNA quantification was performed by calculating the S/C ratio, in which S was the expression level of the target gene and C was the expression of the control gene in hepatic tissue or platelets. If this ratio was >1, the target gene was considered to be overexpressed (OE); otherwise it was under-expressed (UE)

The data were initially analyzed by descriptive statistical techniques and an exploratory analysis was performed to observe the frequency distributions of the variables. Pearson's chi-squared test or Fisher's exact test were used to verify possible associations between the fibrosis degree (G1 and G2); alcohol abuse; and the expression levels of PDGFA, PDGFB, and TGFB1 in the hepatic tissue and platelets. The level of significance for all statistical tests was set at 0.05. Data were analyzed using R software version 3.2.0[14].

A descriptive analysis was performed to evaluate the relative expression levels of the target genes in platelets, using as a control the expression of the same gene in hepatic tissue from the same patient.

Ethical considerations

This study was approved by the Research Ethics Committee of Botucatu Medical School, UNESP (3751-2010). The clinical and demographic characteristics of the patients included in this study are shown in **Table 1**.

TABLE 1

Demographic and clinical characteristics of HCV-infected patients included in this study.

Characteristics	HCV-infected patients, n (%)		P-value
	women	men	
Age, median (IQR)	57 (52.5–61.5)	48 (39–59)	---
Sex	14 (32.6)	29 (67.4)	---
Alcohol abuse[a]			
yes	2 (14.3)	18 (62.1)	0.004
no	12 (85.7)	11 (37.9)	
Fibrosis			
G1 (F1-F2)	7 (50.0)	12 (41.4)	0.745
G2 (F3-F4)	7 (50.0)	17 (58.6)	

HCV: hepatitis C virus; **IQR:** interquartile range; **G1**: group 1 (lower stages of fibrosis - F1 or F2); **G2:** group 2 (higher degrees of fibrosis - F3 or F4). [a]Alcohol abuse was defined as >40g per day for women and >80g per day for men.

When considering TGFB1, PDGFA, and PDGFB expression in the liver tissue and platelets, using the F1 pool as a control, there was a significant association between the fibrosis degree and PDGFA mRNA expression in platelets (**Table 2**). Furthermore, the expression of PDGFA mRNA in platelets was significantly lower in G2 (patients with F3 or F4) than in G1.

When comparing the expression levels of PDGFA, PDGFB, and TGFB1 in platelets and hepatic tissue from the same patient in a descriptive analysis (and the biological significance inherent to variables), the results showed that TGFB1 was upregulated in platelets compared with hepatic tissue in G1 and G2. The opposite was observed for PDGFB, which was downregulated in platelets compared with hepatic tissue in G1 and G2. PDGFA was downregulated in G2 (**Table 3**).

The present study showed that PDGFA mRNA in platelets was significantly downregulated in the advanced fibrosis group when the F1 pool was used as the control. Although previous studies have not described PDGFA mRNA levels in platelets, Thieringer et al.[10] demonstrated that PDGFA mRNA expression was associated with increased TGFB1 protein expression in hepatic tissue from transgenic mice, suggesting a profibrogenic role for PDGFA via induction of the TGFB1 signaling pathway. The lower expression of PDGFA mRNA observed in this study could suggest, for the first time, an association between fibrosis degree and PDGFA expression in platelets.

Although no statistical analysis could be performed as a result of the data distribution, the expression of TGFB1 mRNA in platelets was higher when compared to its expression in hepatic tissue from the same patient in both groups, and PDGFA mRNA was downregulated in G2. The downregulation of PDGFA mRNA in G2 could indicate that this factor was consumed by TGFB1 activation. These findings support the idea that PDGFA and TGFB1 have interrelated functions. Using the same descriptive analysis, the results showed that PDGFB mRNA in platelets was downregulated. This could be a consequence of the accumulation of PDGFB in hepatic tissue, since the PDGFB isoform is considered the most important mitogen for HSCs[7,9] and its overexpression the cause of liver fibrosis in transgenic animals[15].

In conclusion, since platelets accumulate in hepatic tissue during chronic hepatitis C[11] and these cell fragments secrete PDGF, the results of this study suggest an alternative mechanism to the development of fibrosis, in which the activation of HSCs could be performed by PDGFA RNA expression from megakaryocytes as an inducer of the TGFB1 signaling pathway.

TABLE 2

TGFB1, PDGFA, and PDGFB expression in liver tissue and platelets using the F1 pool as the control.

Parameters	Groups of patients with HCV		P-value
	G1 (n=19)	G2 (n=24)	
Alcohol abuse[a]			
no	6	14	0.15[b]
yes	13	10	
PDGFA mRNA in hepatic tissue			
UE	10	10	0.70[b]
OE	9	14	
PDGFA mRNA in platelets			
UE	0[c]	6[c]	0.02[d]
OE	19[c]	18[c]	
PDGFB mRNA in hepatic tissue			
UE	10	8	0.34[b]
OE	9	16	
PDGFB mRNA in platelets			
UE	19	22	0.50[d]
OE	0	2	
TGFB1 mRNA in hepatic tissue			
UE	14	16	0.74[d]
OE	5	8	
TGFB1 mRNA in platelets			
UE	0	2	0.50[d]
OE	19	22	

TGFB1: transforming growth factor beta-1; **PDGFA:** platelet-derived growth factor A ; **PDGFB:** platelet-derived growth factor B; **HCV:** hepatitis C virus; **G1**: group 1 (lower stages of fibrosis - F1 or F2); **G2:** group 2 (higher degrees of fibrosis - F3 or F4); **mRNA:** messenger ribonucleic acid; **UE:** underexpression; **OE:** overexpression. [a]Alcohol abuse was defined as > 40g per day for women and > 80g per day for men. [b]Analysis using Pearson's chi-squared test. [c]Significant association. [d]Analysis using Fisher's exact test.

TABLE 3

Evaluation of PDGFA, PDGFB, and TGFB1 expression in platelets compared with the same genes in corresponding hepatic tissue.

Groups	Genes expressed in platelets					
	PDGFA mRNA		PDGFB mRNA		TGFB1 mRNA	
	UE	OE	UE	OE	UE	OE
G1 (n=19)	4	15	19	0	0	19
G2 (n=24)	10	14	23	1	1	23

TGFB1: transforming growth factor beta-1; **PDGFA:** platelet-derived growth factor A; **PDGFB:** platelet-derived growth factor B; **mRNA:** messenger ribonucleic acid; **G1**: group 1 (lower stages of fibrosis - F1 or F2); **G2:** group 2 (higher degrees of fibrosis - F3 or F4); **UE:** underexpression; **OE:** overexpression.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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