ANNALS of Hepatology

ORIGINAL ARTICLE

March-April, Vol. 12 No.2, 2013: 248-255

Addition of pentoxifylline to pegylated interferon-alpha-2a and ribavirin improves sustained virological response to chronic hepatitis C virus: a randomized clinical trial

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ABSTRACT

Background and aim. The commonly accepted treatment for hepatitis C virus (HCV) infection, pegylated interferon alpha (PEG INF-alpha) and ribavirin, leads to 50-60% of sustained virological response (SVR). On the other hand, pentoxifylline (PTX) possesses antiviral and hepatoprotector properties. **Aim.** To investigate whether the addition of PTX to conventional hepatitis C treatment increases SVR. **Material and methods.** Seventy two patients of both genders were studied in a randomized fashion; the diagnosis of chronic HCV infection was made according to clinical and laboratory criteria and histopathologically classified according to METAVIR scoring system criteria. HCV viral load was tested by PCR, baseline, and after 6 months of treatment, as well as anti-HCV, anti-hepatitis B virus , and anti-human immunodeficiency virus antibodies by enzyme-linked immunosorbent assay. During 48 weeks, control group patients were treated with PEG INF-alpha-2a plus ribavirin. PTX was administered to Experimental Group patients prior to the treatment. **Results.** Demographic data were similar in both groups. Experimental- and control-group subjects were at F2 and F3 states according to the METAVIR classification. The most common HCV genotypes were 1a and 1b (39% in the control group in each case, and 42% in the experimental group in each case). At the end of the study, hepatic enzymes and viral load decreased in both groups to similar values. SVR in the experimental group increased significantly ($p < 0.05$) when compared with standard therapy alone. **Conclusion.** Addition of PTX to conventional chronic hepatitis C treatment may increase the percentage of patients with SVR.

Key words. Chronic hepatitis C virus. Pentoxifylline. Pegylated interferon-alpha-2a. Ribavirin. Sustained virological response.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a public health problem worldwide. According to World Health Organization (WHO) data, 3% of the world population (approximately 170 million persons) is infected with HCV.[1,2] The prevalence of HCV genotypes reported in Mexico is similar in the different regions of the country. The most frequent HCV genotype is 1 (73%) with the following distribution: 1a (28.6%); 1b (37.4%), and 1a/1b (4.1%). The mainly risk factor in Mexico continues to be blood transfusion.[3] At present, in 1a and 1b viral HCV genotypes, PEG INF-alpha 2a or 2b associated with ribavirin is considered the most effective standard therapy for chronic hepatitis C, obtaining SVR rates between 54 and 63%.[4,5] The mechanisms responsible for the progression of liver disease to severe liver injury remain poorly understood. However, there are reports suggesting that oxidative stress (OS) contributes to steatohepatitis and that increased generation of reactive oxygen species (ROS) promotes the development of the hepatic and

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Manuscript received: June 17, 2012.
Manuscript accepted: September 14, 2012.

extrahepatic complications of HCV infection.[6] It has also been reported that Tumor necrosis factor-alpha (TNF-alpha) is required for granuloma formation and is one of the most important cytokines involved in liver injury. These data suggest that TNF-alpha inhibitors may represent a new therapeutic strategy to treat immune-mediated inflammatory liver diseases.[7] The methylxanthine drug pentoxifylline (PTX) has shown antioxidant properties and is considered to be a potent inhibitor of nuclear transcription factor-kappa B (NF-κB),[8] which is a key regulator of genes related to the immune response including regulation of proinflammatory cytokines such as TNF-alpha. Moreover, PTX may reverse human immunodeficiency virus-related endothelial dysfunction by directly inhibiting the endothelial leukocyte adhesion pathway[9] and in combination with vitamin E can ameliorate ribavirin-associated hemolysis.[10] PTX has been proven experimentally to inhibit HIV-1 replication. PTX is also able to protect liver and kidney from the side effects of chemotherapy and radiotherapy.[11]

The effectiveness of the addition of PTX to a conventional treatment with PEG INF-alpha-2a and ribavirin was tested in a prospective controlled blind randomized assigned parallel-group pilot study in patients with HCV chronic infection.

MATERIAL AND METHODS

Study universe

Of 136 patients, we studied 72 patients from the Valentín Gómez Farías State Workers' Safety and Social Security Institution (ISSSTE) Hospital in Guadalajara, Jalisco State, Mexico, from January 2009 through September 2010. These patients were selected randomly by mean of a random numbers table.[12] Forty eight were not included due to other viral genotypes and 16 were eliminated due to breach-of-treatment (Figure 1).

Inclusion criteria

Patients included in the study had chronic hepatitis C virus (HCV) infection diagnosed according to clinical and laboratory criteria[13] (with genotypes 1a, 1b, and mixed 1a/1b), of both genders, between 18 and 70 years of age, and with a diagnosis of HCV confirmed by hepatic enzymes, by determination of anti-HCV antibodies, viral load ≥ 600 International units (IU)/mL, and histological studies according to the METAVIR classification.

Non-inclusion criteria

We did not include pregnant or nursing women, patients with degenerative chronic or hematological disease, decompensated hepatopathy, and patients with terminal-phase ischemic disease.

Exclusion criteria

Patients excluded from the study were those with treatment intolerance, severe hepatic decompensation, platelets < 50,000/mL, and patients who did not comply with 80% of the treatment.

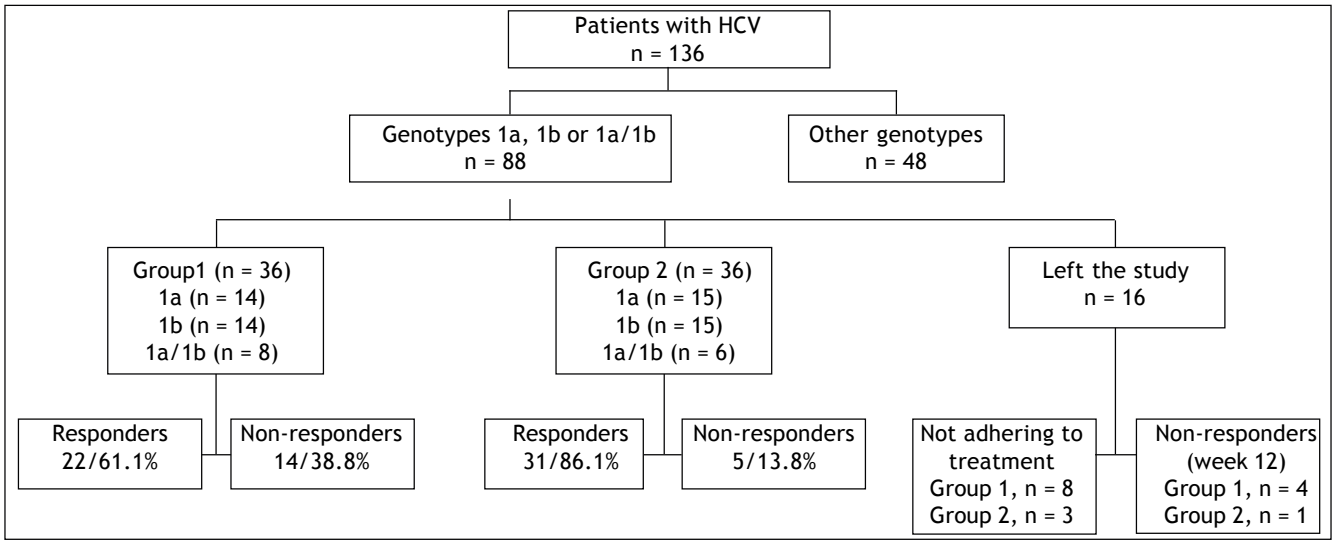

Figure 1. Characteristics of patients with hepatitis virus C (HVC).

Hepatic enzymes

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in the serum of patients by the spectrophotometrically enzymatic automatized Beckman Coulter Synchron method[14] employing kits 442620 and 442665, respectively.

Determination of anti-HCV, anti-hepatitis B virus, and anti-HIV antibodies

HCV was determined utilizing the anti-HCV kit (Abbott 6C37), which detects HCV HCr43, and c100-3 antigens. Anti-hepatitis B virus (HBV) antibodies were detected with the HBsAg kit (Abbott 6C36), while HIV was detected with the HIV Ag/Ab Combo Kit, which detects HIV-1/HIV-2 antibodies and the HIV p24 antigen (Abbott 48-3504/R4). The detection system was based on chemiluminescent microparticle two-step immunoassay employing the ARCHITECT system (Abbott) according to the manufacturer's instructions.

Viral load

Hepatitis C viral load was measured by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor v2.0; Roche Diagnostics, Somerville, NJ, USA) following the manufacturer's instructions. Briefly, 100 μL of serum was subjected to chaotropic lysis in the presence of known amounts of an internal Quantitation standard (QS). The QS comprises a synthetic RNA transcript with primer binding regions identical to those of the HCV target sequence, a randomized internal sequence similar in length and base composition to the HCV target sequence, and a unique probe-binding region that differentiates QS from the target amplicon. After isopropanol precipitation and an ethanol wash, target viral RNA and QS were resuspended in Specimen Diluent (Roche) and this mixture was mixed with an equal volume of the amplification-ready solution (Master Mix; Roche) containing primers KY78 (biotinylated) and KY80 (non-biotinylated), deoxynucleoside triphosphates, AmpErase, and rTth DNA polymerase. Amplification, amplicon dilution, detection, and quantitation were automatically performed by the COBAS AMPLICOR analyzer.[15]

Histology

The patients had an hepatic biopsy by ultrasound or laparoscopy with Tru-Cut® needles. The biopsy slices were stained with hematoxylin and eosin and observed under light microscopy. Degree of fibrosis and histologic activity were scored according to the METAVIR classification.

Protocol

Non previously treated patients were divided into two groups of 36 patients each. Control group (group 1) patients were treated with PEG INF-alpha-2a (180 μg/week, subcutaneous [s.c.]; Roche Laboratories), plus ribavirin (Virazide Grossman Laboratories) with weight-dependant doses (800-1,200 mg per day, administered in two or three doses per os (p.o.). In the experimental group (group 2), pentoxifylline (PTX) (Tentral Aventis Pharma Laboratories) was added at a dose of 400 mg/12 h p.o. to the previously described schedule of PEG INF-alpha-2a plus ribavirin. Both groups were treated during 48 weeks. If a patient did not respond to treatment within a 12-24-week period, he/she was excluded from the study. All tests were carried out at the beginning of treatment and 6 months later at the end of treatment.

Sustained virological response

This is defined as the absence of detectable HVC 24 weeks after the end of the treatment.

Statistical analysis

Results represent the mean ± standard error (SE) between groups; the Mann-Whitney U test was utilized for quantitative variables and the χ^2 test, for qualitative variables; $p \leq 0.05$ was considered significant. In some cases, $\Delta\%$, which represents the percentage of increase or decrease relative to the value found at the beginning of the study, was calculated as follows:

$$\Delta\% = \frac{\text{First determination x 100}}{\text{Last determination}} - 100$$

Ethical considerations

The protocol was authorized by the Institutional Scientific and Ethics Committees and registered with the number ISSSTE/CEI/019/08. All procedures were those strictly used in the management of this pathology and all patients signed an informed consent form.

Table 1. General characteristics of patients with hepatitis virus C (HVC).

Group	Age (years) Range / $\overline{X}$ ± SD	Gender (n / %) Female/Male	Mode of infection (n / %)	Viral genotype (n / %)	Histology (METAVIR) (n / %)	Viral load (IU/mL) $\overline{X}$ ± SD	Treatment response (n / %)
1 (n = 36)	33-69 / 52.1 ± 8.2	28 / 78.0 8 / 22.0	Blood transfusion 26 / 72.2	1a 14 / 39	F1A1 1 / 30	All cases (B) 492,000 ± 26721 (ETR) 97,002 ± 4,792	Responders 22 / 61.1
			Drug abuse 5 / 13.8	1b 14 / 39	F2A2 1 / 30	Responder (B) 208,000 ± 27,966 (ETR) ≤ 600	No responders 14 / 38.8
			Others 5 / 13.8	1a/1b 8 / 22	F3A3 14 / 40	Non responder (B) 284,000 ± 31,016 (ETR) 354,000 ± 42,372	
2 (n = 36)	22-70 / 48.2 ± 12.5	29 / 80.5 7 / 19.5	Blood transfusion 29 / 80.5	1a 15 / 42	F1A1 7 / 20	All cases (B) 519,000 ± 10,381 (ETR) 49,000 ± 4,244	Responders 31 / 86.1
			Drug abuse 3 / 8.3	1b 15 / 42	F2A2 18 / 50	Responder (B) 167,000 ± 16,440 (ETR) ≤ 600	No responders 5 / 13.8
			Others 4 / 11.3	1a/1b 6 / 16	F3A3 11 / 30	Non responder (B) 354,000 ± 42,372 (ETR) 389,000 ± 49,830	
P value	NS	NS	NS	NS	NS	(B) *vs.* (ETR) p = 0.006 All cases (ETR) group 1 *vs.* group 2 p < 0.05	χ^2 = P < 0.01

IU: international units. SD: standard deviation. SE: standard error. *vs: versus.* B: Baseline. ETR: end of treatment response. NS: not significant. METAVIR classification, F1: portal fibrosis; F2: few septa; F3: numerous septa; F4: cirrhosis. A0: without histological activity; A1: minimum activity; A2: moderate activity; A3: severe. Group 1: pegylated interferon (PEG INF)-alpha-2a [180 μg/week, subcutaneously (s.c.) plus ribavirin (800 mg at 1,200 mg/kg/day, divided into two or three doses, *per os* [p.o.])]. Group 2 = pentoxifylline (PTX) (400 mg/12 h, p.o.) plus PEG INF alpha-2a plus ribavirin.

RESULTS

Control group 1, treated with PEG INF-alpha-2a plus ribavirin, was composed of 28 females (78%) and eight males (22%) (mean age, 52.1 ± 8.2 years; range, 33-69 years), while experimental group 2, treated with PTX plus PEG INF-alpha-2a, was composed of 29 females (80.5%) and seven males (19.5%) (mean age, of 48.2 ± 12.5 years; range, 22-70 years). There were no statistical differences in demographic data (Table 1).

Histological study

Seventy percent of control group 1 patients were classified in states F2 and F3 according to the METAVIR classification, while 80% of experimental group 2 patients were classified in these same states (Table 1). Lesions were evident in both groups.

Viral genotype

Viral genotypes (Table 1) were similar in both groups. Control group 1 had 14 cases with genotype 1a and 14 cases with genotype 1b (39% in each case). Experimental group 2 had 15 cases of genotype 1a and 15 cases of genotype 1b (42% in each case). Finally, there were eight cases in control group 1 and six cases in experimental group 2 of 1a/1b genotype (22 and 16%, respectively).

Hepatic enzymes

The hepatic enzymes studied were the following: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and these were determined before and after treatment. At the beginning of treatment, control group 1 had a mean ALT level of 76.7 ± 7.1 International units (IU)/L, and 6 months later at the end of the treatment, this value down to 42.4 ± 5.4 IU/L ($p < 0.001$). Experimental group 2 had serum level values of 67.8 ± 7.3 IU/L, and 6 months later at the end of the treatment, 37.3 ± 3.5 IU/L ($p < 0.01$). There was no difference between both groups either at the beginning or at the end of treatment. The whole population was also divides between no responder and responder patients to the treatment. At the beginning of treatment the ALT level of no responder patients to the treatment was 54.1 ± 7.3 IU/L and for responder patients was 28.1 ± 8.5 IU/L, $\Delta\% = 48.0$ and at the end of treatment the values diminished in no responder subpopulation to 36.3 ± 12.4 IU/L and for responder subgroup the ALT concentration was not modified 23.5 ± 2.4 IU/L. In contrast in experimental group for the first ALT values similar values were observed in no responder and responder patients (31.6 ± 2.9 IU/L and 26.8 ± 2.3 IU, and at the end of study the values for both subpopulations practically were not modified (32.5 ± 3.1 IU/L and 37.3 ± 3.6 IU/L repectively). For the AST enzyme at the beginning of treatment, control group 1 had a serum level of 69.4 ± 5.3 IU/L, and at the end of the study 29.9 ± 3.6 IU/L ($p < 0.01$). At the beginning of the treatment, experimental group shown AST serum values of 67.2 ± 6.3 IU/L, which decreases at the end of treatment 32.5 ± 3.1 IU/L ($p < 0.01$). The values of AST according to no responder and responder patients to the treatment. In relation to control group at the beginning of study similar AST levels were found between no responder 30.8 ± 1.7 IU/L and responder patients 31.6 ± 2.3 IU/L, for the last determination a tendency to increment was observed in no responder patients 51.6 ± 6.4 IU/L and in responder group practically the AST level was not modified 26.9 ± 3.3 IU/L. In the cases of experimental group at the beginning of the study the AST level for no responder group was slightly higher 62.4 ± 7.3 IU/L and in responder subpopulation 41.5 ± 5.4 IU/L, in contrast at the end of the treatment we did not observe differences in both subpopulations group 1: 43.6 ± 5.4 IU/L and group 2: 47.7 ± 5.5 IU/L.

Viral load

Viral load is one of the most important laboratory tests for monitoring HCV. Values at the beginning and the end of the assay 6 months after the treatment are reported in table 1. Initial viral load counts were comparable: control group 1 viremia was 492,000 ± 26,721 IU/mL, and that of experimental group 2'519,001 ± 10,381 IU/mL ($p < 0.05$). At the end of the assay, control group 1 viremia was 97,002 ± 4,792 IU/mL and experimental group 2 viremia was 49,000 ± 4,244 IU/mL, values significantly different from their respective baseline values ($p = 0.006$), $\Delta\%$ was calculated; the decrease in viral load between baseline and final values was $\Delta\% = -407$ for control group 1 and $\Delta\% = -1{,}028$ for experimental group 2, an indication of better behavior of the group of patients who received PTX. In relation to treatment of responders patients from groups 1 and 2 showed a viral load of 208,000 ± 27,966 IU/mL and 167,000 ± 16,440 IU/mL, respectively, and for both groups at the end of the study, the viral load was ≤ 600 IU/mL. In contrast, viral

load values for no responder patients of control group 1 were 284,000 ± 31,016 IU/mL at the beginning and these were practically was not modified at the end of the study, 354,000 ± 42,372 IU/mL. In relation to no responder patients from group 2, viral load values were 352,000 ± 41,525 IU/mL at the beginning and at the end of the study, 389,000 ± 49,830 IU/mL.

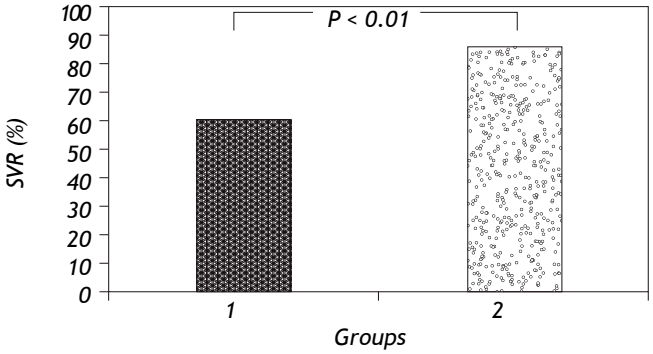

***Figure 2**. Six months after treatment percentage of sustained virological response (SVR) in patiens with chronic hepatitis C. Group 1: PEG-INF-alpha 2a (180 µg/week, s.c.) plus ribavirin (800 at 1,200 mg kg/day, divided in 2 or 3 doses, p.o.). Group 2: pentoxifylline (400 mg/12 h p.o.) plus PEG-INF-alpha 2a plus ribavirin. N = 36 patients/group. Treatments during 48 weeks. χ^2, group 1* vs. *group 2 P < 0.01.*

Sustained virological response

Sustained virological response (SVR) in chronic hepatitis C is considered the gold standard for assessment of treatment response. SVR is obtained when viremia is maintained at a value < 600 IU/mL at 24 weeks after the end of treatment. In control group 1, 22 of 36 patients (61%) achieved SVR *vs.* 31 of 36 patients (86%) in experimental group 2. χ^2 test analysis ($p < 0.01$) between the two groups (Figure 2).

Side effects

Experimental group 2 presented the same side effects as control group 1, although the following were present more frequently: nausea; vomiting; dyspepsia; anorexia, and anemia. However, in every case, the patients did not stop treatment and their side effects were easily controlled (Table 2).

DISCUSSION

In this study, when PTX was added to the conventional PEG INF-alpha-2a plus ribavirin treatment of chronic hepatitis C virus, a higher percentage of SVR was obtained. Both groups had similar demographic and histopathologic characteristics and patients were infected with the same virus types, with a predominance of genotypes 1a and 1b.

Table 2. Side effects of patients treated with either pegylated Interferon (PEG INF)-alpha-2a plus ribavirin (control group 1) or pentoxifylline (PTX) plus PEG INF-alpha-2a plus ribavirin (experimental group 2).

Symptoms	Group 1 (n/%)	Group 2 (n/%)	P value
Flu	5/14	4/11	NS
Asthenia	24/67	22/61	NS
Myalgia	16/44	20/55	NS
Fever	16/44	16/44	NS
Nausea	5/14	24/66	< 0.004
Vomiting	1/3	5/14	< 0.005
Dyspepsia	2/5	15/41	< 0.00002
Anorexia	2/5	7/19	< 0.0008
Anemia	8/22	16/44	< 0.008
Weight loss	10/28	1/39	NS
Thrombocytopenia	3/8	2/4	NS
Hypothyroidism	1/3	-	NS
Depression	4/11	3/8	NS
Sleep alterations	2/5	4/11	NS

During 48 weeks, control group 1 (n = 36) was treated with pegylated interferon (PEG INF)-alpha-2a (180 µg/week, subcutaneously [s.c.]) plus ribavirin (800-1,200 mg/kg/day) and experimental group 2, pentoxifylline (PTX) (400 mg/12 h, per os (p.o.) plus PEG INF-alpha-2a (180 µg /week, s.c.) plus ribavirin (800-1,200 mg/kg/day, p.o.). The data were analyzed by chi-square test. NS: not significant.

ALT and AST serum levels decreased after the conventional and the experimental treatments. Because the experimental treatment introduced an additional drug, greater toxicity was expected; however, hepatic enzyme values were identical, very likely due to the fact that PTX possesses hepatoprotector effects[11,16,17] and exhibits anti-inflammatory and antioxidant properties; in irradiated animals, PTX raised glutathione peroxidase levels, preserving the small intestine mucosa and decreasing acid malondialdehyde, prostaglandin E2 (PGE2), and thromboxane B2, as did the associated PTX treatment, with an important diminution in Nuclear factor-kappa B (NF-κB) and TNF-alpha expression.[17]

Tracing changes in viral load provides the clearest idea of how quickly the infection is progressing. Both treatment schedules were effective in reducing the viral load, this more intensively in the experimental group. However, Δ% calculations clearly showed that the decrease was more important in experimental group 2 because Δ% was then 2.5 times lower than control group 1 values. PTX does not impair the activity of PEG IFN-alpha-2a and ribavirin.

Our results clearly indicate that the addition of PTX to conventional treatment with PEG INF-alpha-2a plus Ribavirin increases SVR by 26% in patients when compared with the conventional treatment ($p < 0.01$), assuming that this difference was due to PTX. In agreement with our results, PTX has also been used with encouraging results in non-alcoholic steatohepatitis, alcoholic hepatitis, and hepato-renal syndrome.[18,19]

On the other hand, every treatment exhibits side effects. In this regard, it is important to consider the following two points: the first is that with the experimental treatment, certain side effects, such as nausea, vomiting, dyspepsia, anorexia, and anemia, were observed more frequently than in patients with the conventional treatment schedule with PEG INF-alpha-2a plus ribavirin. However, these sideeffects were easily controlled and treatment was not discontinued in any group. Thus, it is possible that when the drugs are combined, some properties may be maintained while others may appear. With these results, it is possible to state that the addition of PTX is safe. Recently, we published encouraging results utilizing PTX in the treatment of fulminating C hepatitis.[20] It is also note worthy that other experimental protocols, such as those conducted with protease inhibitors (Telaprevir), reached 70% of SVR, but its side effects are considerable, suchas viral resistance and allergic reactions in 51% of patients in whom 5% were of a severe nature, contrary to PTX, which is described as a nephroprotector.[21]

Study of the PTX action mechanism lies outside of the scope of the present work; however, it is possible that our observations may be due to the antioxidant, antiviral, and anti-inflammatory properties described for this methylxanthine.

Finally, it is important to stress that the "n" of the patients was not extensive and that this work was not blinded. However, despite these facts, we think that these did not play an important role in our observations because despite the "n" of cases between both groups, we are able to observe, clearly and without ambiguity, statistical differences related with SVR. The second reason is due to that determination of SVR is based on laboratory results, minimizing the research effect.

In conclusion, the treatment schedule of HCV with PTX plus PEG INF-alpha-2a plus ribavirin increased the number of patients with SVR by 26% *vs.* the response observed with conventional treatment with PEG INF-alpha-2a plus ribavirin. However, despite the encouraging results described in previous publications,[20] it is necessary to confirm these observations with other *ad hoc* protocols.

ABBREVIATIONS

- **HCV:** hepatitis C virus.
- **HBV:** hepatitis B virus.
- **PEG INF-alpha:** pegylated interferon-alpha.
- **SVR:** sustained virological response.
- **PTX:** pentoxifylline.
- **ALT:** alanine aminotransferase.
- **AST:** aspartate aminotransferase.
- **IU:** international units.
- **TNF-alpha:** tumor necrosis factor-alpha.
- **SD:** standard deviation.
- **SE:** standard error.

ACKNOWLEDGMENTS

We are indebted to Diana Maldonado-Sánchez, B.Sc., for proof reading the manuscript. We also thank our technicians Leticia Ramos-Zavala, María de Jesús Delgado-Ávila, and Marlin Corona-Padilla.

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