Effect of interferon therapy on fatty acid saturation index in hepatitis C virus infection

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أثر العلاج بالإنترفيرون على منسب تشبع الأحماض الدهنية في حالة العدوى بفيروس التهاب الكبد "سي" C.

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الخلاصة: تمت مقايسة نسبة حمض الستياريك إلى حمض الأوليك، أي منسب تشبع الأحماض الدهنية، في أغشية كريات الدم الحمراء، في 60 مريضاً بعدوى فيروس التهاب الكبد سي C المزمن قبل العلاج بالإنترفيرون ألفا وبعده. وبمقارنة النتائج بعشرين من الشواهد الأصحاء، تبيَّن أنه في خلال فترة تتراوح بين شهرين وخمسة أشهر من العلاج بالإنترفيرون ألفا، لوحظ وجود علاقة عكسية بين منسب تشبع الأحماض الدهنية وبين الحِمْل من فيروس التهاب الكبد سي C. ونخلص من ذلك إلى أن العدوى بفيروس التهاب الكبد سي C تزيد من درجة اللاتشبع على الكربون 18 في الأحماض الدهنية، وأن الإنترفيرون ألفا ضالعٌ في استقلاب هذه الأحماض عن طريق زيادة درجة التشبع وما يعقب ذلك من نقص سيولة الأغشية.

ABSTRACT The ratio of stearic to oleic acids, i.e. the fatty acid saturation index, in red blood cell membranes was assayed in 60 patients with chronic hepatitis C virus infection before and after interferon-α therapy. Results were compared with 20 healthy controls. Hepatitis C virus titre was also assayed before and after interferon-α therapy. Within 2–5 months following interferon-α therapy, a significant inverse correlation was observed between saturation index and hepatitis C virus load. We conclude that hepatitis C virus infection enhances the degree of desaturation of 18-carbon fatty acids and that interferon-α is involved in their metabolism by increasing the degree of saturation and subsequent decrease in membrane fluidity.

Effet du traitement par interféron sur l'indice de saturation des acides gras dans l'infection par le virus de l'hépatite C

RESUME Le taux des acides stéariques par rapport aux acides oléiques, c'est à dire l'indice de saturation des acides gras, dans les membranes des globules rouges a fait l'objet de dosages chez 60 patients atteints d'infection chronique par le virus de l'hépatite C avant et après le traitement par interféron-α. Les résultats ont été comparés avec 20 témoins en bonne santé. Le titre du virus de l'hépatite C a également fait l'objet de dosages avant et après le traitement par interféron-α. Dans une période de 2–5 mois après le traitement par interféron-α, une corrélation inverse significative a été observée entre l'indice de saturation et la charge virale de l'hépatite C. Nous concluons que l'infection par le virus de l'hépatite C renforce le degré de désaturation des acides gras contenant 18 atomes de carbone et que l'interféron-α est impliqué dans leur métabolisme par l'augmentation du degré de saturation et la diminution ultérieure de la fluidité de la membrane.

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Introduction

The physical properties of lipids depend on the length of the chain and the degree of desaturation of the constituent fatty acids. A higher content of unsaturated fatty acids increases fluidity of cell membranes, which in turn increases the metabolic rate of many cellular enzymes [*1*]. It has been shown that viruses, interferon and diphtheria toxin can modulate the saturation of the 18-carbon fatty acids (C18 FA), especially conversion of stearic to oleic acid — i.e. the delta-9 (D9)-desaturase system. Interferon and diphtheria toxin inhibit desaturation of stearic acid [*2*], whereas viruses that induce fusogenic syncytia, namely Newcastle disease virus, herpes simplex type 2 and a putative interferon antagonist, promote the D9-desaturase system [*3*].

Chronic liver diseases are frequently associated with abnormalities in lipid metabolism. Merli et al. have shown an absolute increase in unsaturated free fatty acids in plasma and in fatty acid composition of adipose tissue of patients with chronic liver diseases [*4*]. Various studies have demonstrated a consistently higher content of oleic acid relative to stearic acid in peripheral red blood cells of patients with liver cirrhosis and hepatocellular carcinoma [*5*].

The aim of this work is to assess hepatitis C virus (HCV) titre and the saturation index (SI) of red blood cell membranes in patients with HCV infection before and after interferon (IFN) treatment, and to correlate changes in the two parameters after IFN-α therapy.

Methods

To determine the SI of human red blood cells in states of health and chronic HCV infection, blood specimens were collected from 80 patients and 20 healthy controls. The group with chronic HCV infection comprised 60 patients diagnosed by positive polymerase chain reaction for HCV, both qualitatively [*6*] and quantitatively [*7*].

For SI assay, a 5 mL blood sample was collected on EDTA from all participants and centrifuged at 1000 rpm to precipitate red blood cells. These were washed three times with isotonic 0.17M Tris-HCl buffer, pH 7.6, and centrifuged at 1000 g for 20 minutes at 4 °C. The red blood cells were washed twice more after removal of the buffy coats [*8*].

Lipids were extracted from red blood cell ghosts in a tissue grinder with chloroform/methanol (2:1 v/v). After homogenization, the residual protein precipitate was removed by centrifugation and the extract washed with methanol water. Fatty acids were separated from total lipids as the saponifiable fraction, using 0.5 mmol alcoholic potassium hydroxide according to the method described by Folch [*9*]. Fatty acids were recovered by acidification. Methyl esterification was then performed according to the method described by Balint [*10*]. The extracts were analysed by gas/liquid chromatography (Varian, California, United States of America) of the fatty acids' methyl esters (programmed at 170–260, 4/minutes) with a 2.1 mm × 2 m glass column packed with a 3% SP–23/0/ 2% SP–2300 on /00/00/120 mesh chromosorb W (Supelco Incorporated, Pennsylvania, United States of America). Nitrogen was used as the carrier gas at a flow rate of 30 mL/minute. Detection of the fatty acids was by flame ionization detector (Varian).

Stearic acid methyl ester and oleic acid methyl ester were purchased from Sigma and used as external standards. Chromatographic analysis accuracy and certainty were confirmed by using analytical recovery of known added concentrations of both stearic and oleic acid methyl esters. Recov-

ery was 100%. A calibration procedure with pure standards of both oleic and stearic acid methyl esters was applied for chromatographic analysis and calculations using computer software (Varian). The technique was optimized by examining its reproducibility on replicated analysis of samples and standards of different concentrations. Conventional statistical techniques were used to factor out the interassay component of variance, which was 1%–2% throughout most of the analyte concentration range.

Statistical analysis was carried out using *SPSS*, version 5.8. The mean, standard deviation and standard error of mean were calculated. The Rank–Spearman correlation test was applied to assess the relationship between different studied parameters among a group. The probability (P) value, obtained from the statistical table with $(n_1 + n_2 - 2)$ degrees of freedom, was calculated. $P < 0.05$ was considered significant.

Results

Results are summarized in Tables 1–3. Table 1 demonstrates the SI of chronic hepatitis individuals before and after IFN-α therapy. Before IFN-α therapy, chronic hepatitis patients exhibited significant decreases in SI compared to the control group (0.804 ± 0.057 versus 1.501 ± 0.250) ($P < 0.0001$). The values of SI were normalized after IFN-α therapy (1.395 ± 0.117 versus 1.501 ± 0.250). IFN-α significantly increased SI ($P < 0.0001$). Tables 2 and 3 demonstrate HCV titre (copies/mL) in patients in the high and low titre group before and after IFN-α therapy respectively. In patients in the high titre group, IFN-α significantly decreased viral titre from 1 665 200 ± 1 013 417.80 to 279 000 ± 378 857.68 ($P < 0.0001$). Furthermore, there was a significant inverse correlation between the decrease in viral titre and increase in SI after IFN-α therapy in this group ($r = -0.801$, $P < 0.001$). In the low titre group, IFN-α significantly decreased viral titre from 121 866.66 ± 289 413.38 to 108 333.33 ± 189 099.46 ($P < 0.0001$). There was a significant inverse correlation between the decrease in viral titre and the increase in SI after IFN-α treatment in this group ($r = -0.712$, $P < 0.05$). Table 4

Table 1 **Saturation index in chronic hepatitis patients before and after IFN-α therapy**

	Control group	Before IFN-α treatment	After IFN-α treatment	Difference before and after IFN-α treatment (paired *t*-test)
Mean	1.501	0.804	1.395	0.575
s	0.250	0.057	0.117	0.134
$s_{\bar{x}}$	0.055	0.007	0.015	0.017
P		< 0.0001	NS	< 0.0001

IFN = interferon.
s = *standard deviation.*
$s_{\bar{x}}$ = *standard error of the mean.*

Table 2 **Hepatitis C virus titre (copies/mL) in the high titre group patients before and after IFN-α treatment**

	Before IFN-α treatment	After IFN-α treatment	Difference before and after IFN-α treatment (paired *t*-test)
Mean	1 665 200.00	279 000.00	1 201 000.00
s	1 013 417.80	378 857.68	977 225.09
$s_{\bar{x}}$	320 499.00	119 815.83	309 052.84
P			< 0.0001

Viral load ranged from 108 000 to 3 725 000 copies/mL before IFN-α treatment, whereas it ranged from 1000 to 882 000 after IFN-α treatment. There was a significant inverse correlation between changes in viral titre and changes in saturation index after IFN-α treatment in the high titre group (r = –0.801, P < 0.001).
IFN = interferon.
s = *standard deviation*
$s_{\bar{x}}$ = *standard error of the mean.*

Table 3 **Hepatitis C virus titre (copies/mL) in the low titre group patients before and after IFN-α treatment**

	Before IFN-α treatment	After IFN-α treatment	Difference before and after IFN-α treatment (paired *t*-test)
Mean	121 866.66	108 333.33	1 456.666
s	289 413.38	189 099.46	16 631.204
$s_{\bar{x}}$	74 745.19	74 664.11	4 294.158
P			< 0.0001

Range of viral load before IFN-α treatment was 1800–89 000 copies/mL. After IFN-a treatment, the range of viral load was 600–879 000 copies/mL.
There was a significant inverse correlation between changes in viral titre and changes in saturation index after IFN-α treatment in the low titre group (r = –0.712, P < 0.05).
IFN = interferon.
s = *standard deviation*
$s_{\bar{x}}$ = *standard error of the mean.*

shows demographic data of the HCV patients and Table 5 shows the IFN dosage and patient numbers.

Discussion

The regulation of membrane rigidity is essential for homeostasis [*11*]. Decrea-

Table 4 **Demographic data of hepatitis C virus patients**

Parameter	Mean ± *s*	Range
Age (years)	44.3 ± 6.70	34–65
Duration of infection (years)	8.13 ± 1.63	5–10
Duration of treatment (months)	3.46 ± 1.72	2–7
No. of injections	29 ± 22	2–81

s = *standard deviation.*

Table 5 **Interferon dosage and patient numbers**

Interferon dose	No. of patients
3 MU	30
3 MU + virazol	20
5 MU	9
5 MU + virazol	1

sed membrane rigidity leads to increased cellular metabolism and to higher division rates, a feature characteristic of malignant cells [*12*]. The ratio of saturated to unsaturated fatty acids (stearic to oleic), the so-called 'saturation index', reflects the activity of the enzyme D9-desaturase [*5*].

This study shows that in patients with HCV infection, a consistent change is seen in the lipids extracted from red blood cell ghosts. The relative increase in oleic compared with stearic acid indicates enhancement of the normal process of irreversible desaturation of fatty acids with subsequent increase in membrane fluidity. Whereas several studies have demonstrated a significant reversible desaturation of stearic acid in cell membranes in cells infected with viruses such as Coxsacki and herpes simplex type 2 virus [*3*], no previous studies have been conducted to investigate the effect of HCV on red blood cell membrane fluidity. These findings may provide indirect evidence that viral infections may affect biological membrane fluidity.

Mokhov and Bliuzdin [*13*] have reported similar decreases in SI of liver tissue from patients with chronic hepatitis, liver cirrhosis and fatty hepatosis, and Wood [*5*] found a low SI in red blood cells in patients with chronic obstructive jaundice. More recently, Fan and Zhang [*14*] have shown that there is abnormal membrane fluidity of neutrophils in patients with chronic active hepatitis and subfulminant hepatic failure. The altered membrane fluidity is responsible for neutrophil dysfunction in chronic active hepatitis and subfulminant hepatic failure. They also showed that recombinant interleukin-2, one of the cytokines released from Kupffer cells, could significantly increase membrane fluidity.

The reasons for this persistent change in SI are not exactly known. It might be due to impaired metabolism of lipids, substantial regeneration of tissue within the liver, or undetected precancerous lesions, especially in patients with cirrhosis [*5*]. Alternatively, involvement of Kupffer cells in the pathogenesis of liver damage has been suggested [*15*]. Induced Kupffer cells produce a host of cytokines and enzymes, one of which may be D9-desaturase [*16*]. Liver damage is more pronounced due to the high content of hepatic mononuclear cells. Tanikawa and Sata [*15*] have reported that endotoxaemia is found in all kinds of liver injury, including hepatitis C, alcoholic hepatitis and cholestasis. Endotoxin induces Kupffer cells to liberate a group of cytokines, including growth factors such as transforming growth factor-B (TGF-B). Growth factors have been reported to en-

hance the normal process of irreversible desaturation of fatty acids in neoplastic cancer cells and in red blood cell membranes [*17*]. There is direct evidence for increased presence of growth factors in chronic viral hepatitis, with a subsequent increase in biological membrane fluidity [*18,19*]. Zhang et al. [*18*] and Liu et al. [*19*] confirmed that TGF-B could be used as a biochemical prognostic marker for prediction of malignant transformation in chronic viral hepatitis patients. Since growth factors enhance the process of irreversible desaturation of stearic acid, a persistent and significantly high degree of desaturation of stearic acid in chronic hepatitis patients after IFN-α therapy should be evaluated as a prognostic biochemical marker for malignant transformation. Worman [*20*] reported that IFN reduces production growth factors with resulting inhibition of desaturation of fatty acids. This finding explains the observed increase in the SI associated with IFN treatment.

Our results demonstrated a significant increase in the SI 2 months after starting IFN injections in HCV-infected patients. Our findings accord with those of Apostolov and Barker [*21*], who found that IFN increases the SI of cultured human fibroblasts *in vitro*. These results suggest that one effect of IFN on the metabolism of fatty acids is to increase the saturation of C18 FA with a subsequent decrease in membrane fluidity. Furthermore, Worman [*20*] showed that INF normalizes the SI of RBC ghosts in hairy cell leukaemia. A similar finding was obtained by Apostolov and Barker [*3*]. Reduced membrane fluidity results in inhibition of cell division, failure of virus maturation and release of enveloped viruses, and also in virus replication by inhibiting enzymatic activity [*22*].

Our results, showing a significant inverse correlation between changes in viral titre and changes in SI induced by IFN-α therapy, may provide an explanation for IFN's effects on membrane fluidity, which may be caused by its antiviral actions.

Conclusion

From this work, we can conclude that HCV enhances the degree of desaturation of C18 FA, and that IFN-α is involved in the metabolism of C18 FA by increasing their degree of saturation and subsequent decrease of membrane fluidity either directly through D9-desaturase system or indirectly through its antiviral actions. We recommend the use of fatty acid SI of red blood cell ghosts as a diagnostic and prognostic marker for HCV patients before and after IFN-α therapy. Long-term follow-up could be justified for those patients with a persistently high degree of membrane fluidity after IFN-α therapy. A persistently high degree of membrane fluidity should be investigated as a prognostic marker for malignant transformation.

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Hepatitis C

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood.

The major causes of HCV infection worldwide are use of unscreened blood transfusions, and re-use of needles and syringes that have not been adequately sterilized.

The prevalence of HCV infection in some countries in Africa, the Eastern Mediterranean, South-East Asia and the Western Pacific (when prevalence data are available) is high compared to some countries in North America and Europe.

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