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Lavori originali

Original articles

The ursodeoxycholic acid for the treatment of HCV infections

L'acido ursodeossicolico per il trattamento delle infezioni da HCV

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Hepatitis C Virus (HCV), first identified in 1989, is known to be the major cause (> 90%) of sporadic post transfusion Non-A, Non-B hepatitis in western countries [1, 4]. The virus may cause acute and often asymptomatic hepatitis, which may result in either chronic infection in 50% of cases or cirrhosis in 20% of cases. Patients with cirrhosis may develop hepatocarcinoma [5].
Therefore, its evolution has to be blocked, before the irreversible phase. Currently, interferon (IFN) is the only effective therapeutic approach which is likely to modify the natural course of the disease. There are restrictions as to its use (non-compliant patient, severe side effects, simultaneously occurring pathologies, patient age); hence, alternative therapies have been investigated [6].
Ursodeoxycholic acid (UDCA) stands out among non antiviral molecules. Trials conducted in 1987 by Poupon et al. for the treatment of Primary Biliary Cirrhosis [7, 8] and then by other authors for the treatment of sclerosing cholangitis [9, 10, 11] and chronic hepatitis infections both with cholestatic print and viral origin [12, 13, 14, 15], showed a decrease in cytolysis and cholestasis index.
Based upon these findings, ursodeoxycholic acid (UDCA) proved to be effective in reducing serum values of enzymes and inducing a possible variation of viraemia and histologic score over a population of patients with chronic HCV infections, that could not be treated with IFN. This single-blind study reports the results of a treatment with UDCA (600 mg/day for 12 months) compared with a control group.

MATERIALS AND METHODS

The patients enrolled in the above-mentioned randomized stratified trial were selected from a group of subjects with HCV chronic pathology that fulfilled the following criteria.

a) increase in serum ALT levels 3-fold higher than normal values over the previous six months;
b) no other cause of chronic liver disease (viral hepatitis B, Wilson's disease, autoimmune hepatitis, etc.);
c) positivity for AB-HCV (Ortho diagnostic system, Raritan, New Jersey, USA); III generation confirmatory test (Ortho); HCV-RNA (Amplicor HCV PCR Kit, Roche molecular systems, Basel, Switzerland);
d) histologic pattern of either chronic hepatitis or compensated cirrhosis, according to international standard criteria;
e) no previous antiviral treatment;
f) exclusion of subjects with anti-HIV positivity and drug addicts.
74 patients, 51 males and 23 females, mean age 47.9 years (range 25-71) were investigated; histological examination indicated that 38 subjects were affected by CAH, 27 by CPH and 9 by cirrhosis. HCV typing was also studied, according to Simmond's classification; 29 patients belonged to genotype 1a, 34 to genotype 1b, 11 to genotype 2a [16].

THERAPEUTIC PROTOCOL

Patients were randomly assigned to two stratified groups of 37 subjects, each having the same age, sex, pathology stage and genotypes. Patients recruited for this single-blind trial were submitted to the following therapeutic protocol:
1) Group A: 37 patients (26 males and 11 females; means age 47.7 yrs; 19 subjects were affected by CAH, 13 by CPH, and 5 by cirrhosis), treated with UDCA (600 mg/day given in two administrations for twelve months);
2) Group B: 37 patients (25 males and 12 females; mean age 48.1 yrs; 19 subjects were affected CAH, 14 by CPH and 4 by cirrhosis) treated with placebo for twelve months.

Table 1 - Characteristics of patients included in the treatment.

	A	*B*
No. of patients	37	37
Female (no.)	11	12
Mean age (years)	47,7	48,1
Jaundice	absent	absent
Presence of HCV-RNA	37	37
Genotypes:		
1a (no. of pts)	15	14
1b (no. of pts)	17	17
2a (no. of pts)	5	6
CAH	19	19
CPH	13	14
Cirrhosis	5	4

The level of pre-treatment serum enzymes in patients of both groups was superimposable (166.8±32.2 versus 169.2±37.2). All patients gave informed consent (Tabella 1).

LABORATORY TESTS AND HISTOLOGY

All patients were laboratory monitored at the 3rd, 6th, 9th, 12th month both during treatment and after the 12-months' follow-up with complete check up: total hemochrome, transaminase, gGT, alkaline phosphatase.
HCV-RNA was detected at the 3rd, 6th and 12th months of treatment and at the 6th and 12th month of follow-up.
The control biopsy at the end of the follow-up was performed only in those patients who normalized ALT values.

DETECTION OF HCV-RNA

For the Amplicor HCV PCR kit, we followed the manufacturer's instructions. Briefly, RNA was extracted from 100 µl serum with the lysis buffer containing guanidine thiocyanate and 2-mercaptoethanol in the presence of RNA carrier; RNA was then recovered by isopropanol precipitation. HCV RNA was reversely transcribed and amplified in a single-tube reaction using a ready-to-use master mix containing Tth DNA polymerase, AmpErase, primers KY80 (sense 5' GCA GAA AGC GTC TAG CCA TGG CGT 3') and KY 78 (antisense 5' CTC GCA AGC ACC CTA TCA GGC AGT 3') [11] containing a biotin residue at the 5' terminus to allow detection of amplification product. The conditions for RT-PCR were as follows: one cycle at 50 °C for 2 min, 60 °C for 30 min, 95 °C for 1 min; two cycles at 95 for 15 s, 60 °C for 20 s; 38 cycles at 90 °C for 15 s, 60 °C for 20 s; one cycle at 60 °C for 4 min; samples were soaked at 72 °C until use. After alkaline denaturation, amplification products were hybridized to probe KY88 (5' GGT GGG TCG CGA AAG GCC TTG TGGT 3') [11] immobilized in wells of microtiter plates. After the addition of avidin-peroxidase conjugate and chromogen substrate, the absorbance was read at 450 nm. Samples showing optical density (OD) values exceeding 0.4 were considered as positive. Negative and positive controls were included in each run (4 controls for each set of 20-25 clinical samples).

STATISTICAL METHODS

Wilcoxon's test was used for the statistical analysis. Results were reported as mean ± S.E.M. The statistical significance is given by $p<0.05$.

RESULTS

Thirteen patients (8 of group A, 5 of group B) did not complete the study. Two patients for pregnancy, three for chronic diarrhea (group A), one because of change of residence and seven for non-compliance.
A reduction of AST and ALT levels was observed in 19 patients (65.5%) of group A, with mean values of 43.7±27.1 and 58.3±30.8 IU/L ($p<0.01$) at the end of the treatment. Eight patients showed a normalization of transaminase (complete response), while eleven subjects showed a 40% reduction (partial response); such evolution occurred during the 3rd month of therapy and remained steady in 15 patients for the whole treatment. In 4 subjects, two with a complete response and two with partial response, the transaminase levels returned to higher values (88.2±7.5 and 92.4±13.5 IU/L) though they were lower than pre-treatment values.
The cholestasis index (ALP-GGT) showed a rate parallel to that of transaminase, with a return to normal values in subjects with complete or partial response (ALP: 145.5±34.7; GGT: 35.6±11.3). It is interesting to note that a ALP

Table 2 - Serum enzymes mean levels during treatment and follow-up.

		Baseline	*3rd month*	*6th month*	*9th month*	*12th month*	*18 month*
AST	Gr A	145,7±15,6	93,6±15,6 a	47,2±12,5 a	39,6±19,3	43,7±27,1 b	147,9±10,1 a
	Gr B	146,1±10,9	128,7±15,1	131,6±15,7	146,4±18,1	64,4±16,4 b	143,4±13,6 b
ALT	Gr A	166,8±32,2	105,6±23,4 c	63,5±25,8 a	59,3±22,1	58,3±30,8 c	171,3±29,6 a
	Gr B	169,2±37,2	153,6±17,6	146,6±21,2	148,6±17,2	71,5±26,6	169,3±18,4
GGT	Gr A	58,8±10,5	36,4±11,2 a	33,4±10,4 a	32,6±19,2	35,6±11,3 a	44,1±7,6 a
	Gr B	57,5±10,9	52,4±6,2	56,2±10,2	60,1±15,3	52,2±7,4 c	52,3±13,7
ALP	Gr A	225,3±18,3	189,4±31,7	191,4±29,7 b	148,6±31,4	145,5±34,7 a	159,2±28,4 c
	Gr B	225,3±12,8	206,6±30,5	217,4±32,3	133,2±24,5	211,8±20,5	217±31,2
		Normal values:	AST/ALT 5-40 IU/L		GGT 10-40 IU/L		ALP 60-270 IU/L
		a p<0,001	b p<0,01	c p<0,05			

and GGT reduction was also reported in non-responders. These results did not differ significantly according to the hepatopathy stage or to genotype. In group B only 4 patients (12.5%) showed a remarkable and steady reduction of enzymes (AST: 64.4±16.4; ALT: 71.5±26.6 IU/L) (p:n.s.) and a contemporary decrease in ALP and GGT values (211.8±20.5 and 52.2±7.4).
The follow-up emphasized a return of serum AST and ALT levels to pre-treatment values (147.9±10.1; 171±39 IU/L) (p<0.01) in 17 patients (69.5%) belonging to group A between the 2nd and the 4th month after discontinuing UDCA.
Despite an increase during the follow-up, in group A the cholestasis index did not reach pre-treatment values. It appeared to be steadily 35% lower than baseline values (Tabella 2).
Nineteen patients (15 of group A and 4 of group B), all responders, accepted to undergo a control biopsy, but nobody showed any improvement in the histologic picture.

EFFECTS ON SERUM HCV-RNA

All patients were still viremic at the end of the treatment. The optical density (OD) remained steady with absorbance values between 1.8-2.4.

DISCUSSION

The decision to use bile acids in chronic liver and biliary disease is based on the belief that retention of endogenous hydrophobic cytotoxic bile acids can play an important role in this disease [17]. *In vitro* studies have shown that elevated liver perfusion of these acids causes cell membrane rupturing with spilling of transaminases. When bile ducts are blocked or narrowed these bile acids deposit within liver cells and cause damage [18, 19].
It has been reported that the more bile acids react with surface membranes, are lipophilic and therefore detergent (solubilize lipids), the more they are toxic. Based on this a descending liver toxicity scale is lithocholic > deoxicholic > chenodeoxicholic > cholic > ursodeoxicholic acid [18-20].
In humans UDCA treatment is useful since liver cells take up and excrete about 6-10 grams per day of bile acids. Most are lipophilic. Thus UDCA treatment modifies the endogenous pool of these acids and increases the urso rate by 35-50%. As a result the detergent action lowers because of rapid elimination in the bile and increased competition between other bile acids and UDCA at the site where intestinal reabsorption occurs [19-21]. The mechanism used by UDCA to bring about changes in cell lysis and

bile duct blockage appears to be more complicated; several potential explanations may be given.
1) The membrane stabilizing effect with reduced permeability [22].
2) The inhibited rise in alkaline phosphatase synthesis occurring when bile acids lower after common bile duct blockage [23].
3) The increased excretion of liver enzymes in bile and decreased return to the blood stream since UDCA protects small canal tight-junctions [24].
4) Reduction of antigen HLA expression (class 1) on biliocytes and on hepatocytes with subsequent decrease in the toxic effect of T lymphocytes [25].
5) The effect of UDCA as an immune modulator, possibly reducing liver autoimmune damage which may initiate with chronic alcoholism [26].
Experience gained from literature and the above observations have paved the way to this study.
Results obtained can be regarded as satisfactory, since the proportion of patients, who showed a reduction in serum ALT levels, after UDCA treatment, was remarkably higher than the control group ($p<0.05$). In particular, in 8 patients a complete response was reported at the 3rd month of treatment. Values remained steady during the whole therapy treatment; in 11 subjects the response was partial, but only in 4 patients values were found to be twice as normal values).
The basic borderlines of the UDCA are the following: non-alteration of the histologic score, no variation of viremia, drug-dependent effect on cytolysis index given the absence of long-term improvement after the treatment interruption [27]. During the follow-up it was noted that, within 4 month from the interruption of UDCA, about 69% of responders exhibited AST and ALT values similar to pre-treatment values. Its action on the cholestasis index is different. In this case the response was steady and values remained lower that the pre-treatment levels, thus confirming that the efficacy of UDCA is stronger in hepatitis infections with prevalent cholestatic print.
As to the outcome of UDCA treatment in patients affected by viral chronic hepatitis, no change was reported in the natural history of the disease. However, this molecule may be a valuable alternative for patients who cannot be treated with antiviral drugs (IFN, Ribavirin, etc.). The mechanism of action of UDCA, though ineffective on the initial damage, can block the evolution of the cell cholestasis-mediated damage, as showed by the improvement in cytolysis index in a wide range of patients.

Key words: Ursodeoxycholic acid, HCV infections.

RIASSUNTO

L'epatite da HCV, in assenza di trattamento specifico, progredisce verso l'epatite cronica e verso la cirrosi epatica rispettivamente nel 50% e nel 20% dei casi.
Il trattamento di scelta sembra a tutt'oggi essere quello dell'interferone, ma tale terapia non può essere effettuata i tutti i pazienti per la scarsa compliance, l'elevato numero di effetti collaterali anche severi oltre che per numerose controindicazioni (età, patologie associate, neutro e piastrinopenia...).
L'acido ursodeossicolico (UDCA) è stato studiato da numerosi autori come possibile agente non antivirale alternativo all'IFN nel trattamento dell'epatite cronica da HCV.
Nel presente studio "single blind" sono stati arruolati 74 pazienti affetti da epatite cronica da HCV.
Un gruppo di 37 pazienti è stato trattato con UDCA (600 mg/die per 12 mesi), un altro gruppo di 37 pazienti (controllo) con placebo.
Tredici pazienti dei due gruppi di trattamento non hanno completato il ciclo di terapia: il 65,5% dei pazienti appartenenti al gruppo UDCA hanno mostrato una riduzione dei valori di AST (nel 42% con normalizzazione completa). I valori di AST rimanevano immodificati in 15 pazienti di tale gruppo.
Nel gruppo controllo invece solo il 12,5% dei pazienti hanno mostrato una significativa riduzione dei valori di AST. Nessuna variazione è stata osservata nella viremia e/o negli indici istologici nei due gruppi considerati.
L'acido ursodeossicolico sembra essere un utile trattamento per ridurre gli indici di citolisi epatica nel pazienti con epatite cronica da HCV, è ben tollerato, ma non sembra modificare il corso naturale della malattia.

SUMMARY

Hepatitis C is a very serious disease. If it is not treated, it leads to chronic hepatitis and cirrhosis in 50% and 20% of cases, respectively. Furthermore, patients with cirrhosis might develop hepatocarcinoma.
Interferon seems to be the therapy of choice in the treatment of the disease. However, since the molecule cannot always be used (non-compliant patient; severe side effects; liver-associated pathologies; patient age) alternative therapies have been investigated. Ursodeoxycholic acid (UDCA) stands out among recommended non-antiviral molecules.
74 patients affected by HCV chronic infections were enrolled in this single-blind study. One group (A: 37 patients) was treated with UDCA (600 mg/day for 12 months) and compared with a control group (B: 37 patients) in order to assess the therapy efficacy in reducing cytolysis index and to estimate viremia and histologic score variation.
Results: 13 patients did not complete the study (8 belonging to group A; 5 to group B). 65,5% of the patients treated with UDCA showed ALT reduction; 42,1% of them with complete response. The situation remained unchanged in 15 patients all the treatment along. In group B, only 12,5% showed a significant ALT reduction. During the follow-up, in 69,4% of group-A responders ALT was found to return to pretreatment values. No variation was observed in the viremia and histologic score of patients who had accepted a control biopsy.
Conclusions: UDCA is undoubtedly suitable for reducing cytolysis index in patients with HCV chronic infections. It is well tolerated but it does not modify the disease natural course.

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