Treatment of active chronic hepatitis with cyclophosphamide

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Summary

Twenty patients with active chronic hepatitis, classified on the basis of the treatment into three groups, have been studied: (1) patients treated with cyclophosphamide (10 patients); (2) patients treated with cyclophosphamide after cycles of corticosteroid therapy (four patients); (3) patients treated with corticosteroid (six patients).

In all patients we observed clinical, biochemical and immunological changes during and after treatment.

Hepatic needle-biopsy was performed in all patients before treatment. The histological picture was rechecked after the first cycle of therapy in twelve patients.

The results obtained in the three groups of patients have been statistically compared.

Cyclophosphamide treatment produces an improvement of general conditions and a persistent normalization of several biochemical and immunological data, especially in those patients in which active chronic hepatitis was not in an advanced stage (type A aggressive form). The histological picture showed no changes after treatment.

Introduction

Numerous immunological and histological data have given rise to the assumption that active chronic hepatitis (ACH) is closely related to autoimmune phenomena (MacKay, Taft and Cowling, 1956; Popper, Paronetto and Schaffner, 1965; MacKay, Weiden and Hasker, 1965). Nevertheless, it is not known if these immunological changes are the cause or the effect of the disease.

Some cases of ACH seem strictly related to previous acute viral hepatitis, mainly hepatitis-associated antigen (HAA) positive (Sutnick, London and Blumberg, 1971; Naccarato *et al.*, 1972). It is still uncertain whether or not the persistence of a virus in the organism, and above all in the liver, can

imply pathogenetic importance in the development of the disease.

The problem of the treatment of ACH remains unsolved at the present time.

In the last years, in relation to the autoimmune assumptions in the pathogenesis of the disease, the use of immunosuppressant drugs was emphasized. The results obtained are still incomplete and fragmentary.

The most widely used immunosuppressant drugs are the steroid derivatives. This treatment, applied during the early active phase of ACH (2-3 years), reduces the mortality rate and produces an improvement in the general condition of the patients treated. Corticosteroid treatment has also a significant effect in raising serum albumin and in reducing total serum globulins (MacKay and Wood, 1962; Cook, Mulligan and Sherlock, 1971).

Other immunosuppressant drugs, such as 6-mercaptopurin and azathioprine, sometimes in association with corticosteroids, are used, but the efficiency of these therapies is still under discussion. The results obtained were often contradictory. However, some authors observed an improvement in the clinical and biochemical pictures, mainly in moderate ACH, using combined prednisone-azathioprine therapy (MacKay, Weiden and Ungar, 1964; Solway *et al.*, 1970; Mistilis and Blackburn, 1967).

The mechanism of action of immunosuppressive agents is not completely known. They seem to suppress the proliferation of the immunoreactive lymphocytes and to have possible anti-viral effects (Page, Condie and Good, 1964).

Treatment may be followed by some side-effects (leucopenia, thrombopenia, gastric intolerance, infections), and these drugs can have a hepatotoxic action (Kravitz *et al.*, 1967; Mistilis and Blackburn, 1967).

Although the importance of the immunological phenomena in the pathogenesis of ACH is not fully explained at present, we maintain that immunosuppressant treatment must be undertaken in the active stage of this disease.

Cyclophosphamide (Cy), an alkylating agent, used for long periods of time in several diseases (cancer chemotherapy, especially Hodgkin's disease and other malignant reticuloses), can suppress a variety of immunological phenomenon in experiments with animals and in observations on human beings (Santos, 1967; Nakamura and Weigle, 1970; Steinberg *et al.* 1971; Paterson, Drobish and Biddick, 1971; Alepa, Zvaifler and Sliwinski, 1970; Booth and Able, 1970; Grushkin *et al.*, 1970).

The hepatotoxic action of this drug is doubtful. It is present in neither the experimental nor in the clinical use (Grushkin *et al.*, 1970; Lavin and Koss, 1971).

The immunological suppressive action of Cy is not entirely related to the cytotoxic action, like 6-mercaptopurin and azathioprine (Santos, 1967; Nakamura and Weigle, 1970; Alepa *et al.*, 1970; Grushkin *et al.*, 1970; Paterson, Drobish and Biddick, 1971).

Long-term treatment with this drug has no devastating side-effects although cases of sterility after Cy treatment have recently been reported (Fairley, Barrie and Johnson, 1972).

Biological effects of Cy require its microsomal oxidation and its conversion into at least two cytostatic alkylating agents, one of which is nitrogen mustard. This phenomenon appears to take place in the liver (Brock and Hohorst, 1963).

For all these reasons we decided to use this drug in the treatment of the active stage of ACH. This therapy was carried out discontinuously, as is the normal procedure.

We treated with Cy one group of patients who had no previous immunosuppressant therapy and a second smaller group who had had previous corticosteroid treatment. The results obtained in these two groups of patients were compared first with each other and then with results obtained from a third group treated with corticosteroid therapy alone.

All the patients showed immunological and histological findings indicating that the disease was active (increase of serum immunoglobulins, presence in the blood of anti-smooth muscle and anti-glomerular basal membrane antibodies; lymphomononuclear infiltration of the portal bile tracts and along the liver cells, piecemeal necrosis of the liver cells).

Immunosuppressant therapy was not used in the absence of these findings.

We did not leave any patients without treatment for use as controls as the mortality rate is higher and the general condition worse in those patients with ACH without any therapy (Cook *et al.*, 1971).

This study has been performed with the purpose of verifying the efficiency of the cyclophosphamide treatment in active chronic hepatitis.

Patients and therapeutic outlines

We have studied twenty patients with ACH, classified on the basis of the treatment into three groups:

Group 1. Patients treated with cyclophosphamide (Cy) (ten patients).

The outline of the therapy in these patients was: (a) 200 mg daily i.v. up to a total of 5–6 g; (b) suspension of the treatment for 1–3 months; (c) 100–150 mg daily orally or i.v. up to a total of 4–5 g; (d) suspension of the treatment for 1–3 months; (e) possible resumption of the therapy with 100–150 mg daily orally or i.v. up to a total of 4–5 g.

The therapy was applied with weekly check-ups (or more frequently, if necessary) of WBC, RBC, platelets. This therapy was changed or stopped if and whenever leucopenia, thrombopenia or other important side-effects appeared. These patients were clinically checked for 6–18 months.

Group 2. Patients treated with Cy after cycles of corticosteroid therapy (Cy after St) (four patients).

These patients were treated, 3–24 months before the beginning of Cy treatment, with courses of corticosteroid therapy (15–20 mg prednisolone daily in courses of 2–3 months). The subsequent treatment with Cy was applied according to the above mentioned outline. These patients were clinically checked for 5–16 months.

Group 3. Patients treated with corticosteroid therapy (St) (six patients).

These patients, checked for 8–24 months, were treated with cycles of corticosteroid therapy (15–20 mg of prednisolone or 16–24 mg daily of triamcinolone in cycles of 2–3 months).

All the patients of the three groups were admitted to the hospital for 45–60 days. Afterwards, we prescribed rest and a quiet life, possibly at home, in addition to the therapy.

The diagnosis of ACH was established on the basis of clinical, biochemical, immunological and histological data (Tables 1 and 2).

Methods

In all the patients the development of the clinical, biochemical and immunological picture was recorded during and after the treatment. Data after the beginning of therapy was recorded at intervals of 1, 2, 4, 7–9, 10–12, 13–15, 16–18 and 24 months.

Hepatic needle-biopsy was performed in all the patients before the treatment. The histological picture was re-checked after the first cycle of the therapy in twelve patients. The histology was appraised in accordance with the criteria for classification established by De Groote *et al.* (1968).

The clinical data studied were: asthenia, dyspepsia, arthromyalgias, alteration of bowel movements and of diuresis. In examination we looked for the

TABLE 1. Clinical and histological data of twenty patients with active chronic hepatitis (ACH) treated with cyclophosphamide (Cy), with cyclophosphamide after steroid therapy (Cy after St), and with steroids (St)

	Cy ten patients	Cy after St four patients	St six patients
Clinical data			
Sex: males	2	2	4
females	8	2	2
Age: < 30 years	4	2	3
> 30 years	6	2	3
Appearance of the disease: evident	3	2	3
insidious	7	2	3
Date of appearance of illness:			
< 1 year	3	2	4
> 1 year	7	2	2
History: hepatitis	3	2	3
alcohol	5	3	3
other (drugs, biliary tract infectious, etc.)	3	1	1
Associated illnesses	4	2	4
Malaise, nausea	10	3	5
Jaundice	5	3	3
Hepatomegaly	10	4	6
Splenomegaly	4	2	6
Oedema, ascitis	2	2	3
Histological data			
Chronic aggressive hepatitis type A	8	—	3
Chronic aggressive hepatitis type B	2	4	3
with cirrhosis	2	2	1
without cirrhosis	—	2	2

TABLE 2. Humoral and immunological data of twenty patients with ACH

	Cy ten patients	Cy after St four patients	St six patients
Humoral data:			
Serum bilirubin > 1 mg% ml	8	2	4
S-GOT > 40 UW/ml	6	3	6
S-GPT > 40 UW/ml	6	3	6
S-OCT > 100 U/ml	6	2	3
Serum albumin < 3.6 g%	10	4	6
Serum γ -globulins > 1.8 g%	9	4	4
Presence in the serum of Hepatitis Associated Antigen	3	2	3
Immunological data:			
Serum immunoglobulins:			
Serum immunoglobulins:			
IgG > 1600 mg%	6	4	4
IgA > 400 mg%	4	2	1
IgM > 140 mg%	7	4	3
Presence in the serum of:			
anti smooth muscle antibodies	10	4	5
anti glomerular basal membrane antibodies	10	4	5
anti mitochondrial antibodies	8	3	1
anti nuclear antibodies	0	0	0
anti γ -globulins antibodies	6	1	4

presence or not of jaundice, acne, spider naevi, the presence and the extent of hepatomegaly and splenomegaly, and the presence of vascular fragility.

Laboratory investigations performed were: serum bilirubin (Malloy and Evelyn, 1937), serum GO and GP transaminases (Wroblenski and Le Due, 1956),

serum ornithine carbamyl transferase (OCT) (Ceriotti and Gazzaniga, 1966), serum total albumin and gamma globulins, BSP test, serum hepatitis-associated antigen (HAA) (Bedarida, Trinchieri and Carbonara, 1969; Shulman and Barker, 1969).

Immunological data considered were: serum

immunoglobulins (IgG, IgA, IgM) (Mancini *et al.*, 1965), serum antinuclear, anti-smooth muscle, anti-glomerular basal membrane, anti-mitochondrial (Coons and Kaplan, 1950) and anti-gamma globulins antibodies.

All the patients had an oesophaged X-ray to look for varices, and an hepatic scintiscan. These radiological investigations were re-checked after 6 months in ten patients.

Results

Clinical investigations

In the three groups of patients there was a gradual decrease in the asthenia and dyspepsia, a decrease or disappearance of the arthromyalgias, a return to normal of bowel movements and diuresis. The improvement was greater in patients treated with Cy (group 1) than in those of the groups 2 and 3.

Patients treated with Cy showed swift regression or disappearance of the jaundice (if present) within the first month of treatment, while in the other two groups this decrease was slower or absent. Cutaneous findings of chronic hepatitis (acne, vasculo spiders) were emphasized in the first group with the passing of time, while in the other two groups there were no changes. Hepatomegaly, observed in all the patients before the beginning of the treatment, remained unchanged in nine subjects treated with Cy alone, whereas it decreased in the others. A remarkable reduction of the hepatomegaly and at the same time an increase of the splenomegaly was noticed in three patients, one of each group. The splenomegaly, if present, remained practically the same, except the three mentioned patients. Peripheral oedema, ascites, vascular fragility findings (when present) were reduced or disappeared in the first group of patients, while they remained unchanged or increased in the other two groups.

Laboratory investigations

Total serum bilirubin (Fig. 1), serum GO (Fig. 1) and GP transaminases, serum OCT receded permanently into the normal range within 2 months from the beginning of the Cy treatment. Periodic variations of these mean level findings in the other two groups were observed. Serum albumin and serum gamma globulins showed similar behaviour in the three groups of subjects. The mean levels of these findings had periodic variations. Variation of the serum HAA titre can be demonstrated in the HAA positive patients. Treatment with Cy increased the serum HAA titre in some cases.

Immunological data

Serum immunoglobulins G (Fig. 2), A, M levels and the frequency with which the serum anti-smooth muscle, anti-glomerular basal membrane,

anti-gamma globulins antibodies may be found, showed periodic variations in the three groups of patients. Anti-mitochondrial antibodies disappeared from the blood within 12 months in the patients treated with Cy (Fig. 3). No patient showed serum antinuclear antibodies.

Histological picture

Table 1 shows the frequency of the type A and B (with or without cirrhosis) in the three groups of patients. Hepatic needle-biopsy was repeated after the first course of therapy in twelve subjects (eight of group 1, three of group 2, one of group 3). In all cases the histological picture showed no changes when compared with the initial conditions. The findings of piecemeal necrosis, degeneration, and hepatocellular necrosis were no worse. The marked fat degeneration of the liver cells observed in two subjects (one of group 1 and one of group 2) was considerably reduced.

Radiological investigations

Before the beginning of the treatment, oesophageal varices were present in one of the patients of group 1, two of group 2 and one of group 3. In these four patients there was subsequent oesophageal bleeding. Oesophageal varices were not brought to light in the other subjects. Oesophageal X-rays were repeated in ten patients after 6 months, without alterations in the radiological picture.

Hepatic scintiscanning, performed in all the patients and re-checked after 6 months in ten cases, showed reduction of the size of the liver and simultaneous enlargement of the spleen in one patient of each group. In the other patients it remained unchanged.

Statistical analysis

Table 3 shows the statistical analysis of the results obtained in the three groups of patients. The same table explains the comparison of the results obtained with the Cy treatment with those obtained with Cy after cycles of corticosteroid therapy and after corticosteroids alone.

Complications and mortality

In two subjects treated with Cy, who showed severe leucopenia, the treatment was reduced or suspended. One patient of each group had oesophageal haemorrhage and consequently died. Portal-caval anastomosis was performed on a fourth patient (of group 2) and the recovery was satisfactory.

The other side-effects (hair loss in five patients, acne or herpes in four, arthrytis in one, scyatalgia in one) were reduced or disappeared after the end of the cycles of treatment.

Current status of survivors

Current status of survivors is summarized in Table 4.

Discussion

The results obtained in a group of patients with active chronic hepatitis treated with cyclophos-

phamide were compared with those from two other groups of patients treated either with the same drug after cycles of corticosteroid therapy, or with corticosteroids alone.

The Cy treatment leads to a swift improvement in the clinical picture (subjective and objective findings). There is either no improvement or it is slower when

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FIG. 2. Behaviour of IgG in twenty patients with ACH. ●—● Cy; ■—■ St-Cy; ▲—▲ St.

FIG. 3. Behaviour of some immunological findings in twenty patients with ACH. ●—● Cy; ■—■ St-Cy; ▲—▲ St.

TABLE 3. Statistical analysis of the results concerning twenty patients treated with cyclophosphamide and/or with steroids after 1–24 months from the beginning of the therapy

		Cy	Cy after steroids	Steroids	Cy/Cy after steroids	Cy/steroids
<i>S. Total Bilirubin</i>		n.s.	n.s.	n.s.	after 4 m (0.05)	n.s.
<i>S.GOT</i>	↓after	1 m (0.05) 2 m (0.005) 4 m (0.01) 9 m (0.02) 15 m (0.05)	n.s.	n.s.	n.s.	after 2 m (0.01)
<i>S.GPT</i>	↓after	2 m (0.01) 4 m (0.01) 15 m (0.05)	↓after 4 m (0.01) 9 m (0.05)	↓after 18 m (0.02)	n.s.	after 2 m (0.01) 4 m (0.05)
<i>S.OCT</i>		n.s.	n.s.	n.s.	n.s.	n.s.
<i>S. Albumin</i>	↑after	12 m (0.02)	n.s.	n.s.	n.s.	after 12 m (0.02)
<i>S. γ-globulins</i>	↓after	9 m (0.01) 12 m (0.05)	n.s.	↓after 2 m (0.05)	after 9 m (0.025)	n.s.
<i>S.IgG</i>	↓after	9 m (0.05)	n.s.	n.s.	after 9 m (0.025)	n.s.
<i>S.IgA</i>		n.s.	n.s.	n.s.	n.s.	n.s.
<i>S.IgM</i>		n.s.	n.s.	n.s.	n.s.	n.s.

n.s., not significant; significant differences (*P*) are between parentheses; m, months.

TABLE 4. Current status of patients with ACH treated with Cy and/or steroids after 12–24 months of observation

	No. of patients treated	No. of patients alive	Cause of death in the deceased patients	General condition of the patients living	Liver serological tests	Immunological data	Histological data
Cy	10	9	bleeding from oesophageal varices	excellent: 4 subjects good: 5 subjects	normal: 6 subjects abnormal: 3 subjects	normal: 6 subjects abnormal: 3 subjects	unchanged: 8 subjects
Cy after St	4	3	bleeding from oesophageal varices	excellent: 1 subject good: 1 subject bad: 1 subject	normal: 1 subject abnormal: 2 subjects	normal: 1 subject abnormal: 2 subjects	unchanged: 3 subjects
St	6	5	bleeding from oesophageal varices	excellent: 1 subject good: 4 subjects	normal: 3 subjects abnormal: 2 subjects	normal: 3 subjects abnormal: 2 subjects	unchanged: 1 subject

the Cy therapy is applied after corticosteroid treatment or when steroid treatment alone is followed.

Several of the biochemical data (bilirubinaemia, serum GOT, GPT, OCT) often normalize permanently only after the first cycle of Cy treatment. They show periodic variations with alternate reduction and increase outside the normal levels with the other therapies undertaken.

Albuminaemia and gamma globulinaemia, and some immunological findings (serum immunoglobulins, serum anti-smooth muscle, antglomerular basal membrane, anti-gamma globulins antibodies) in the three groups of patients show periodically variations during the progression of the disease. In all the patients treated with Cy, the serum mitochondrial antibodies disappear from the blood.

The histological picture, re-checked in twelve cases after the first course of Cy, did not show any significant change when compared with the conditions before the treatment.

Oesophageal X-rays and hepatic scintiscans showed no significant changes when re-checked after 6 months from the beginning of the Cy therapy.

Three patients—one from each group—died from oesophageal bleeding. A fourth patient (of group 2) also affected by this complication, was successfully operated of porta-caval anastomosis. Marked leucopenia was found in two patients treated with Cy. Other Cy side-effects (loss of hair, herpes, scyatalgia) were quickly reduced or disappeared completely during the period of interruption of this therapy.

Statistical analysis showed the significant improvement of several biochemical findings with Cy treatment (re-entry within the normal range of serum GOT, GPT, OCT, increase of serum albumin and loss of gamma globulins levels). Any significant favourable effect in the behaviour of these findings was obtained with Cy after steroid treatment or with steroid therapy. Nevertheless the comparison

between the Cy group and the other two groups of patients did not show remarkably significant differences.

These preliminary results concerning the effects of Cy treatment in ACH and the comparison of these results with those obtained with Cy after steroids and with steroid treatments, lead to the following considerations.

The dose of Cy used is not hepatotoxic. Therapy is not accompanied or followed by important side-effects.

Cy treatment leads to swift improvement of the general condition and permanent return to normal of several biochemical and immunological data, especially in those patients in which ACH was not in an advanced stage.

However, the majority of the immunological findings show, in spite of this treatment, periodical variations between the normal and the pathological levels. It is not clear if this fluctuation of the immunological findings could be explained on the basis of the discontinuity of the treatment. The disease has periodic variations depending on the degree of activity.

Cy treatment gives the best results in patients with ACH when the condition has moderate immunological and histological levels of activity of the disease. In these patients the histological picture is, in most cases, that of chronic aggressive hepatitis type A.

Generally the results are very poor when this treatment is undertaken in the ACH with serious immunological and histological activity findings (chronic aggressive hepatitis type B, mainly with cirrhosis), even after other therapies have been used.

The preliminary results may be confirmed by further studies.

Some questions remain unanswered.

There is in some patients a contrast between the swift improvement of the clinical and biochemical picture and the persistence—in spite of the immunosuppressant treatment—of abnormal findings of activity of the disease (increase of serum immunoglobulins, persistence in the blood of anti-smooth muscle, anti-glomerular basal membrane, anti-gamma globulins antibodies). The effect of the Cy therapy on the serum HAA is not clear as the first course of treatment increased the HAA titre in some cases.

The speed of the improvement in the general condition, the return of working capacity, the normalization of several biochemical features, the non-progressivity of the histological picture observed in the histologically type A aggressive form ACH patients treated with Cy, are the bases on which we propose to undertake this therapy at this stage of the disease.

On the other hand, the results obtained with Cy

treatment in the histologically type B aggressive form ACH patients gives preference to other therapies—such as corticosteroid treatment—in this advanced stage of the disease. In the advanced stage of the disease, Cy probably gives poor results because it cannot be developed into its active form by the greatly damaged liver.

Further observations are necessary to estimate the long-term effects of cyclophosphamide treatment in active chronic hepatitis.

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