Cancer Biotherapy & Radiopharmaceuticals
Volume 16, Number 2, 2001
Mary Ann Liebert, Inc.

Ciclofosfamide più somatostatina, bromocriptina, retinoidi, melatonina e ACTH nel trattamento dei linfomi non-Hodgkin di basso grado in stadio avanzato: risultati di una sperimentazione di fase II.

Mauro Todisco, MD, Piergiorgio Casaccia, MD e Nazzareno Rossi, ASL 13 in Sondrio e ASL 106 in Teramo.

Scopo

E' stato dimostrato che la somatostatina, la prolattina, i retinoidi, la melatonina e l'ACTH influenzano la crescita linfatica, e l'azione del ciclofosfamide nei disordini linfoproliferativi è ben nota. Questo fornì le basi per condurre, in pazienti con linfomi non-Hodgkin di basso grado (NHL), una sperimentazione di fase II di un'associazione combinata di ciclofosfamide, somatostatina, bromocriptina, retinoidi, melatonina e ACTH.

Pazienti e metodi

Venti pazienti con una diagnosi di NHL di basso grado, di stadio III o IV, furono inclusi in questo studio. I pazienti ricevettero per un mese il seguente trattamento: ciclofosfamide, somatostatina, bromocriptina, retinoidi, melatonina e ACTH. La terapia fu continuata per altri due paesi in pazienti con malattia stabile e rispondente. Dopo tre mesi, i pazienti che rispondevano alla terapia la continuarono per tre mesi e più.

Risultati

Venti pazienti furono valutabili per tossicità e risposta; 70% (14 di 20 pazienti; 95% di intervallo di confidenza [CI], da 50% a 90%) ebbero una parziale risposta; il 20% (4 su 20) ebbero una malattia stabile, e il 10% (2 su 20) progredirono con la terapia. Continuando con la terapia, nessuno dei 14 pazienti con una risposta parziale ebbe una progressione della malattia (tempo di controllo medio di 21 mesi, gamma, da 7 a 25), e il 50% di questi pazienti ebbe una completa risposta e il 75% (3 di 4) progredirono con la terapia (tempo medio di progressione [TTO] 14.3 mesi, gamma, da 7 a 21). La tossicità era molto modesta, gli effetti collaterali più comuni furono: sonnolenza, diarrea e iperglicemia.

Conclusioni

L'associazione di ciclofosfamide, somatostatina, bromocriptina, retinoidi, melatonina e ACTH è ben tollerata ed efficace nel trattamento del NHL di basso grado ad uno stadio avanzato.

INTRODUZIONE

Di recente è stato dimostrato che nuovi agenti inibiscono la crescita linfatica. Tra questi, soprattutto quelli senza tossicità per il midollo osseo, come la somatostatina, suscitarono grande interesse per la possibilità di utilizzarli con regimi di chemioterapia mielosoppressivi senza determinare un ulteriore mielosoppressione.

Un'associazione come questa fu proposta per la prima volta nel 1979 da Di Bella e altri, che utilizzarono il ciclofosfamide insieme con la somatostatina, la bromocriptina, i retinoidi, la melatonina e l'ACTH in diversi tipi di cancro, inclusi i linfoma non-Hodgkin (NHL).

Sulla base di questa associazione farmacologica, in particolare dell'uso della somatostatina e della bromocriptina, esiste l'assunto, formulato da Di Bella, che l'ormone della crescita (GH) e la prolattina sono coinvolti nella crescita neoplastica. Tale assunto, nella leucemia linfoblastica acuta, fu formulato anche da altri autori, nello stesso periodo, anche se solo per il GH. Successivamente, Payan e altri dimostrarono che la somatostatina inibisce la crescita di linfociti umani primari in coltura e di cellule Molt-4; Nakamura e altri identificarono i recettori della somatostatina sulla membrana di diverse linee di cellule linfoidi e Hiruma e altri scoprirono i recettori della somatostatina sulle cellule umane di leucemia primaria. Inoltre, fu dimostrato che la maggior parte delle lesioni linfomatose sono identificabili con gli analoghi radiomarcati della somatostatina.

In accordo con i risultati sopra menzionati, Witzig e altri nel 1995 affermarono che l'octreotide, un analogo della somatostatina, mostra attività in pazienti con il NHL di basso grado. L'influenza sulla crescita linfatica è stata anche dimostrata per la prolattina, i retinoidi, la melatonina e l'ACTH. È stato dimostrato che la prolattina stimola la crescita dei linfomi sperimentali, sia in vivo che in vitro, e i recettori della prolattina sono presenti sulla superficie delle cellule linfoidi neoplastiche e normali. Matera e altri dimostrarono che la prolattina è una fattore di crescita autocrino per una linea di cellule leucemiche umane, e Hooghe e altri ritornarono all'ipotesi, già sostenuta da Di Bella, che la prolattina e il GH hanno un ruolo importante nel linfoma e nella leucemia. In ematologia, l'effetto antitumorale dei retinoidi è basato su diverse prove dell'effetto dell'acido trans retinoico nella leucemia promielocitica, nel linfoma delle cellule T localizzato nella pelle e anche nei linfomi delle cellule B.

La melatonina inibisce l'incorporazione di timidina nei normali linfociti e nelle linee di cellule linfoblastoidi e inibisce la risposta proliferativa ai fitogeni. Inoltre la melatonina porta avanti un azione antimielodisplastica e diminuisce la tossicità per il midollo osseo degli agenti chemioterapici. E' stato dimostrato che i linfociti T e B presentano il recettore dell'ACTH sulla superficie delle loro cellule; inoltre l'ACTH abbassa la blastogenesi dei linfociti in risposta alla fitoemaglutinina e alla concanavallin A e ha un ruolo nella modulazione dell'attività delle cellule NK.

Questi studi, insieme alla ben nota azione del ciclofosfamide, fornirono la base per disegnare uno studio di fase II per determinare se una terapia combinata di ciclofosfamide,

somatostatina, bromocriptina, retinoidi, melatonina e ACTH ha un'attività in pazienti con il NHL di basso grado in stadio avanzato.

PAZIENTI E METODI

Selezione dei pazienti

I pazienti furono selezionati sulla base di una diagnosi clinica di NHL di basso grado, stadio III o IV. Criteri addizionali di selezione furono: uno stato di esecuzione (PS) tra 0 e 3, secondo l'Eastern Cooperative Oncology Group, e la presenza di lesione misurabile bidimensionalmente, come dimostrato dall'esame fisico, dalla radiografia del torace, dall'ecotomografia, dalla tomografia computerizzata o risonanza magnetica.

I pazienti che avevano ricevuto altri trattamenti furono inclusi in questo studio solo se il primo trattamento non era stato efficace. Ai pazienti che ricevevano la chemioterapia fu chiesto di sospendere la somministrazione di qualunque farmaco per almeno 15 giorni prima di iniziare la terapia combinata.

La tossicità fu valutata usando criteri sviluppati dall'Organizzazione Mondiale per la Sanità.

Trattamento

I pazienti ricevettero una combinazione di ciclofosfamide, somatostatina, bromocriptina, retinoidi, melatonina e ACTH. Il ciclofosfamide fu somministrato oralmente ad una dose di 75 mg/giorno (50 mg alle 14:00 e 25 alle 21:00). La somatostatina fu somministrata sottocute (SQ) ad una dose di 1,5 mg/giorno entro 8 ore usando una siringa a pompa. La somministrazione iniziò almeno tre ore dopo la cena e quei pazienti che erano psicologicamente incapaci di accettare questo tipo di somministrazione ricevettero una singola iniezione SQ di octreotide (0,5 mg/giorno) tre ore dopo la cena.

La bromocriptina fu somministrata oralmente ad una dose di 2,5 mg/giorno (1,25 mg alle 14:00 e alle 21:00). I retinoidi: Acido trans retinoico, palpitato di vitamina A e betacarotene furono somministrati oralmente, alle 8:00 in 5 ml di vitamina E, rispettivamente in dosi di 5 mg, 5000 UI e 20 mg/giorno. La melatonina fu somministrata oralmente ad una dose di 20 mg/giorno (10 mg alle 14:00 e alle 21:00). L'ACTH fu somministrato intra muscolo ad una dose di 1 mg/settimana.

Tutti i pazienti furono trattati per almeno 1 mese. Alla fine di questo periodo, quelli che avevano una malattia stabile o una parziale risposta ricevettero altri due mesi di trattamento, e quelli che risposero dopo tre mesi furono trattati per tre mesi e più.

Criteri per la risposta

Una risposta completa o remissione fu definita come la completa regressione di tutte le lesioni linfomatose misurabili. Una risposta parziale fu definita come una riduzione >=50% nella somma dei prodotti dei due diametri (il diametro più lungo e quello ad esso perpendicolare) di una o più lesioni durate almeno 4 settimane.

La progressione fu definita dall'aumento nel volume delle lesioni preesistenti di almeno il 25%, l'insorgere di nuove lesioni o l'aumento della milza e del fegato di almeno 2 centimetri dovuto al linfoma.

Quei pazienti che non potevano essere chiaramente collocati nelle categorie descritte venivano definiti in condizioni stabili. La valutazione della risposta fu fatta a 1 mese dall'inizio del trattamento. La valutazione fu portata avanti dopo altri due mesi e, più tardi, dopo 3 mesi nei pazienti che continuarono il trattamento.

RISULTATI

Venti pazienti (dieci uomini e dieci donne tra i 37 e i 70 anni) con istiologia di basso grado furono inclusi in questo studio, e furono tutti valutati per tossicità e risposta. Il 60% (12 su 20) ebbero istiologia centrocitica-centroblastica e 10 erano stati trattati in precedenza. Il 20% (4 di 20) ebbero istiologia centrocitica, il rimanente 20% ebbe istiologia linfocitica e 3 di 4 in entrambi i gruppi erano stati trattati prima di questo studio. L'8% dei pazienti (16 di 20) erano in uno stadio IV e 20% (4 di 20) erano in stadio III. Il 50% (8 su 16) dei pazienti trattati in precedenza ebbero un periodo in cui non ricevettero la terapia (TFT)>=6 mesi e furono in recidiva; il 50% (8 su 16), con TFT<=1.5 mesi, ebbe una progressione durante la terapia seguita prima di entrare in questo studio. I risultati del trattamento dopo un mese sono descritti analiticamente nella Tabella 1.

Table 1. Response to regimen after 1 month

Age	*Sex*	*Histology*	*Stage*	*PS*	*PT*				*TFT months*	*Response*		
67	F	Centrocytic	IV	3	–	–	–	–	–	PR		
39	M	CC-CB	IV	0	–	–	–	–	–	PR		
64	F	Lymphocytic	IV	2	–	–	–	–	–	PR		
67	F	CC-CB	IV	1	–	–	–	–	–	PR		
48	F	CC-CB	III	0	SC	–	–	–	16	PR		
51	M	Lymphocytic	IV	0	SC	–	–	–	12	PR		
50	F	CC-CB	IV	1	CC	Rt	–	–	24	PR		
62	M	CC-CB	III	0	CC	Rt	–	–	10	PR		
37	F	CC-CB	IV	0	CC	Inf	–	–	36	PR		
58	F	CC-CB	III	0	CC	Inf	–	–	48	PR		
48	F	CC-CB	III	0	CC	SC	–	–	60	PR		
44	F	CC-CB	IV	2	CC	Inf	–	–	6	PR		
40	M	CC-CB	IV	1	SC	Inf	–	–	0,5	PR		
70	M	Lymphocytic	IV	1	Inf	–	–	–	0,5	PR		
51	F	Centrocytic	IV	2	SC	CC	–	–	1,5		SD	
66	M	Lymphocytic	IV	1	SC	–	–	–	0,5		SD	
66	M	CC-CB	IV	1	CC	CC	–	–	1,5		SD	
54	M	Centrocytic	IV	2	CC	HC	–	–	1		SD	
62	M	Centrocytic	IV	1	CC	–	–	–	0,5			Prog
68	M	CC-CB	IV	3	CC	Rt	CC	–	1			Prog

Abbreviations: PT, previous therapies, from left to right in temporal succession; SC, single agent chemotherapy; CC, combination chemotherapy; HC, high dose intensive chemotherapy; Rt, radiotherapy; Inf, interferon; CC-CB, centroblastic-centrocytic; PR, partial response; SD, stable disease; Prog, progression.

Il 70% dei pazienti (14 su 20; 95% CI, da 50 a 90%) ebbero una parziale risposta; il 20% (4 su 20; 95% CI, da 2.5% a 37.5%) ebbero una malattia stabile e il 10% (2 su 20) progredirono nella terapia. La risposta dopo un mese nei pazienti suddivisi secondo le terapie ricevute in precedenza è descritto nella Tabella 2.

Table 2. Response to regimen after 1 month; patients subdivided according to the previously received therapies

NHL	*Number of patients*	*Response (number of patients)*		
		PR	*SD*	*Prog*
Previously untreated patients	4	4	–	–
Previously treated patients				
Patients with TFT≥6 months in first relapse	8	8	–	–
Patients with TFT≤1.5 months non-responding or progressed during interferon therapy	2	2	–	–
Patients with TFT≤1.5 months non-responding or progressed during single agent or combination chemotherapy	6		4	2
All the patients	20	14	4	2

Abbreviations: PR, partial response; SD, stable disease; Prog, progression.

Il 100% (4 su 4) dei pazienti non trattati in precedenza, 100% (8 su 8) dei pazienti con TFT>= 6 mesi e il 100% dei pazienti con TFT>=1.5 che non risposero o progredirono durante la terapia d'interferone, ebbero una parziale risposta. Tra i pazienti con TFT <=1.5 mesi che

non risposero o progredirono durante la chemioterapia con un singolo agente o combinata, il 66.6% (4 su 6; 95% CI, da 28% a 104%) ebbero una malattia stabile e il 33.3% (2 su 6) progredirono. La risposta alla continuazione del trattamento è descritta analiticamente nella Tabella 3.

Table 3. Response to the treatment prosecution

Age	*Sex*	*Histology*	*Response*	*TTP (months)*	*Follow-up (months)*
67	F	Centrocytic	SD	–	25
39	M	CC-CB	CR	–	21
64	F	Lymphocytic	CR	–	21
67	F	CC-CB	SD	–	7
48	F	CC-CB	SD	–	21
51	M	Lymphocytic	SD	–	21
50	F	CC-CB	CR	–	21
62	M	CC-CB	CR	–	21
37	F	CC-CB	SD	–	9
58	F	CC-CB	SD	–	22
48	F	CC-CB	CR	–	19
44	F	CC-CB	CR	–	18
40	M	CC-CB	CR	–	22
70	M	Lymphocytic		–	19
51	F	Centrocytic	PR	–	15
66	M	Lymphocytic	Prog	21	–
66	M	CC-CB	Prog	15	–
54	M	Centrocytic	Prog	7	–

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; Prog, progression; TTP, time-to-progression.

Tra i 14 pazienti che dopo un mese ebbero una risposta parziale, non ci fu progressione della malattia (tempo medio di controllo di 21 mesi, gamma, da 7 a 25), e il 50% di questi pazienti (7 su 14; 95% CI, da 24% a76%) ottennero una completa risposta. Tra i 4 pazienti che dopo un mese ebbero una malattia stabile, il 25% (1 su 4) ebbero una risposta parziale e il 75% (3 su 4) progredirono (tempo medio di progressione 14.3 mesi, gamma, da 7 a 21). La risposta al proseguimento del trattamento nei pazienti suddivisi secondo le terapie ricevute in precedenza è descritta nella Tabella 4.

Table 4. Response to the treatment prosecution; patients subdivided according to the previously received therapies

NHL	*Number of patients*	*Response (number of patients)*			
		CR	*PR*	*SD*	*Prog*
Previously untreated patients	4	2	–	2	–
Previously treated patients					
Patients with TFT≥6 months in first relapse	8	4	–	4	–
Patients with TFT≤1.5 months non-responding or progressed during interferon therapy	2	1	–	1	–
Patients with TFT≤1.5 months non-responding or progressed during single agent or combination chemotherapy	4	–	1	-	3
All the patients	18	7	1	7	3

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; Prog, progression.

Tutti i 20 pazienti furono valutabili per la tossicità. Gli effetti collaterali più comuni furono segni gastrointestinali. 1125% dei casi ebbe diarrea di primo grado (2 pazienti) o secondo grado (3 pazienti); il 20% (4 pazienti) ebbero nausea o vomito di grado 1, e 5 pazienti ebbero perdita di appetito o anoressia. Questi effetti collaterali non richiesero la sospensione della terapia ma solo un aggiustamento della dose di somatostatina. Sonnolenza fu osservata nel 20% (4 su 20) dei pazienti e richiese un aggiustamento del programma giornaliero della somministrazione della melatonina (20 mg/al giorno furono suddivisi in tre dosi, invece che due, una delle quali prima di andare a dormire).

Il 25% dei pazienti (5 su 20) ebbe iperglicemia di grado 1 (<=160/dL) e il 20% (4 su 20) ebbe edema della caviglia e/o della faccia. In entrambi questi casi la dose di ACTH fu ridotta a 0.5 mg/settimana.

DISCUSSIONE

Le NHL di basso grado in stadio avanzato sono ancora malattie incurabili, il cui trattamento, proprio a causa di questi limiti terapeutici, è molto discusso, dato che coesistono la chemioterapia con un singolo agente, la chemioterapia combinata, una combinazione di radioterapia e chemioterapia, la chemioterapia intensiva con dosi massicce con trapianto di midollo osseo autologo o trapianto di cellule progenitrici autologhe.

In questo studio abbiamo valutato la tossicità ed efficacia di un regime, noto come multiterapia Di Bella (DBM), che è un'associazione di un agente chemioterapico, il ciclofosfamide, con altre sostanze non mielosoppressive (somatostatina, bromocriptina, retinoidi, melatonina e ACTH).

I risultati che abbiamo ottenuto - 70% di risposta parziale dopo 1 mese, il 50% dei quali rispose completamente continuando il trattamento - sono stati nettamente migliori rispetto a quelli descritti con un singolo agente chemioterapico con alkylants, con il quale Kimby e altri descrissero il 36% di risposta totale con una risposta completa nel 5% di 132 pazienti trattati in precedenza, o rispetto a quelli descritti da Witzig e altri con la somatostatina come unico agente (36% di risposta parziale in 28 pazienti trattati e non in precedenza). Ciò dimostra la superiorità terapeutica di una tale associazione farmacologia rispetto ai suoi singoli costituenti.

Inoltre, l'attività del regime dipendeva dal tipo di terapia precedente e dal TFT. Abbiamo documentato il 100% di risposta globale tra i pazienti non trattati in precedenza, i pazienti in prima recidiva con TFT>= a 6 mesi e i pazienti con TFT <= 1.5 che non avevano risposto o erano progrediti durante la terapia d'interferone.

Questo risultato, migliore di altri ottenuti con regimi di chemioterapia ampiamente utilizzati [Kimby e altri descrissero il 60% di risposta globale con CHOP in 127 pazienti non trattati in precedenza con NHL di basso grado in stadio III e IV], e l'ottima tolleranza del DBM

(tutti i pazienti continuarono il trattamento a casa, continuando le loro normali attività), suggeriscono ulteriori esperimenti clinici usando questo regime in NHL.

Con riferimento a tutto questo, la recente disponibilità di formule di deposito di somatostatina possono permettere una migliore applicabilità di questo regime, nel quale il principale problema era la somministrazione SQ giornaliera di somatostatina, soprattutto se effettuata con una siringa a pompa.

CANCER BIOTHERAPY & RADIOPHARMACEUTICALS
Volume 16, Number 2, 2001
Mary Ann Liebert, Inc.

Cyclophosphamide plus Somatostatin, Bromocriptin, Retinoids, Melatonin and ACTH in the Treatment of Low-grade Non-Hodgkin's Lymphomas at Advanced Stage: Results of a Phase II Trial.

Mauro Todisco*, MD, Piergiorgio Casaccia, MD, and Nazzareno Rossi
ASL I3 in Sondrio and ASL 106 in Teramo (Local Health Department of National Health Service)

Purpose

Somatostatin, prolactin, retinoids, melatonine and ACTH have been shown to influence the lymphatic growth, and the action of the cyclophosphamide in lymphoproliferative disorders is well known. This provided the rationale to conduct, in patients with low-grade non-Hodgkin's lymphomas (NHL), a phase II trial of a combined association of cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin and ACTH.

Patients and methods

Twenty patients with a diagnosis of low-grade NHL, stage III or IV, were included in this study. Patients received for one month the following treatment: cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin, and ACTH. The therapy was continued for two additional months in patients with stable or responding disease. Afler three months, the responding patients continued the therapy for three months and more.

Results

Twenty patients where assessable for toxicity and response; 70% (14 of 20 patients; 95% confidence interval [CI], 50% to 90%) had a partial response; 20% (4 of 20) had stable disease, and 10% (2 of 20) progressed on therapy. Going on with the treatment, none of the 14 patients with partial response had a disease progression (average follow-up time of 21 months, range, 7 to 25), and 50% of these patients had a complete response; among 4 patients with stable disease, 25% (1 of 4) had a partial response and 75% (3 of 4) progressed on therapy (mean time to progression [TTP] 14.3 months, range, 7 to 21). The toxicity was very mild, the most common side effects being drowsiness, diarrhea and hyperglycemia.

Conclusions

The association of cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin, and ACTH is well tolerated and effective in treatment of low-grade NHL at advanced stage.

* Addres reprint requests to Mauro Todisco, MD, via Leonardo Da Vinci 25, 63013 Grottammare (AI'). Phone:00390735736315 — Fax: 00390735731154- Emai!: todmau@tin.it

INTRODUCTION

Recently, several new agents bave been shown to inhibit the lymphatic growth. Among these, especially the ones without bone marrow toxicity, as somatostatin, aroused great interest for the possibility to be used with myelosuppressive chemotherapy regimens without determining a further myelosuppression.

An association like this was first reported in 1979 by Di Bella et al [1], who reported to have used the cyclophosphamide together with somatostatin, bromocriptin, retinoids, melatonin and ACTH in several cancers, including non Hodgkin's lymphomas (NHL).

On the basis of this pharmacological association and, in particular, of the use of somatostatin and bromocriptin, there is the assumption, formulated by Di Bella, that growth hormone (GH) and prolactin are involved in neoplastic growth. Such an assumption, in acute lymphoblastic leukernia, was formulated by other authors also, in the same period, even if just for GH [2]. Subsequently, Payan et al demonstrated that somatostatin inhibits the growth of cultured primary human liymphocytes and Molt-4 cells [3]; Nakamura et al identified somatostatin receptors on the membrane of several lymphoid cell lines [4] and Hiruma et al found somatostatin receptors on primary leukemia human cells [5] Furthermore, most of lymphomatous lesions were shown to be identifiable with radiolabelled somatostatin analogs. [6-10]

In agreement with the above mentioned results, Witzig et al in 1995 reported that octreotide, a somatostatin analog, shows activity in patients with low-grade NHL [11] The influence on lymphatic growth has been also demonstrated for prolactin, retinoids, melatonin and ACTH. The prolactin has been shown to stimulate the growth of experimental lymphomas both *in vivo* and *in vitro*, [12] and prolactin receptors are present on the surface of normal and neoplastic lymphoid cells.[13-16] Matera et al demonstrated that prolactin is an autocrine growth factor for a human leukemic cell line, [17] and Hooghe et al returned on the hypothesis, already mantained by Di Bella, that prolactin and GH have an important role in lymphoma and leukemia. [18] In hematology, the antitumor effect of retinoids is based on several evidences reporting the effect of trans-retinoic acid in promyelocytic leukemia, [19-20] T-cell lymphoma localized to the skin [21-26] and also in B- cell lymphomas. [27-29]

Melatonin inhibits thymidine incorporation in normal lymphocytes and in lymphoblastoid cell lines [30] and inhibits the proliferative response to mitogens. [31-32] In addition, melatonin carries on an antimyelodysplastic action [33] and decreases the bone marrow toxicity of chemotherapeutic agents. [34] T and B lymphocytes have been shown to express the ACTH receptor on their cell surface [35]; moreover, ACTH depresses the lymphocyte blastogenesis in response to phytoemagglutinin and concanavallin A [36] and has a role in the modulation of NK celi activity. [37]

These studies, together with the well known action of cyclophosphamide, provided the rationale to design a phase II study to determine if a combined therapy based on

cyclophosphamide, somatostatin. brinocriptin, retinoids, melatonin and ACTH has activity in patients with low-grade NHL at advanced stage.

PATIENTS AND METHODS

Patient Selection

Patients were selected on the basis of a clinical diagnosis of low-grade NHL, stage III or IV. Additional criteria of selection weree: a performance status (PS) between 0 and 3, according to the Eastern Cooperative Oncology Group, and the presence of bidimensionally measurable lesion, as demonstrated by physical examination, chest radiograph, ecotomography, or computed tomographic or magnetic resonance scans.

Patients who had received other treatment were included in this study only upon evidence that the previous treatment was not effective. Patients receiving chemotherapy were asked to suspend any drug admnistration for at least 15 days prior to the beginning of the combined therapy.

Toxicity was evaluated using criteria developed by the World Health Organization.

Treatment

Patients received a combination of cyclophosphamide, somatostatin, bromocriptin, retinoids, melatonin and ACTH. Cyclophosphamide was given orally at a dose of 75 mg/day (50 mg at 2 pm and 25 al 9 pm). Somatostatin was administreted subcutaneously (SQ) at a dose or 1.5 mg/day within 8 hours using a syringe pump. The administration started at least three hours after dinner and those patients who were psychologically unable to accept this type of administration received single SQ injection of octreotide (0,5 mg/day) three hours after dinner. Bromocriptin was given orally at a dose of 2.5 mg/day (1.25 mg at 2 pm and at 9 pm). Retinods: all-trans retinoic acid, vitamin A palmitate and beta-carotene were administered orally, at 8 am, in 5 ml of vitarnin E, respectively at doses of 5 mg, 5000 UI and 20 mg/day. Melatonin was given orally at a dose of 20 mg/day (10 mg at 2 pm and at 9 pm). ACTH was administered intermuscularly at a dose of 1 mg/week.

All the patients were treated for at least one month. At the end of this period, those who had stable disease or partial response received additional two months of treatment, and the ones who responded after three months were treated for three months and more.

Criteria for Response

Complete response or remission was defined as the complete regression of all the measurable lymphomatous lesions. Partial response was defined as a ≥ 50% reduction in the sum of the products of the two diameters (the longest diameter and the one perpendicular to it) of one or more lesions lasting at least 4 weeks.

Progression was defined by the increase in size of the pre-existing lesions of at least 25%, the onset of new lesions or the increase of spleen or liver of at least 2 centimeters due to lymphoma.

Those patients who could not be clearly placed in any of the described categories were defined in stable condition. The assessment of the response was made after 1 month from the beginning of the treatment. Such an assessment was carried on after another 2 months and, later, every 3 months in patients going on with the treatment.

RESULTS

Twenty patients (ten males and ten females between 37 and 70 years old) with low-grade histology were included in this study, and all were assessable for toxicity and response. Sixty percent (12 of 20) had centroblastic-centrocytic histology and 10 were previously treated. Twenty percent (4 of 20) had centrocytic histology, the remaining twenty percent had lymphocytic histology and 3 of 4 in both groups had been treated prior to this study. Eighty percent of the patients (16 of 20) were stage IV and 20% (4 of 20) were stage III. Fifty percent (8 of 16) of the previously treated patients had a therapy-free time (TFT) ≥ 6 months and were in relapse; 50% (8 of 16), wilh TFT ≤ 1.5 months, had a progression during the therapy followed before being enrolled in this study. The results of the treatment after 1 month are analitically described in Table 1.

Table 1. Response to regimen after 1 month

Age	*Sex*	*Histology*	*Stage*	*PS*	*PT*				*TFT months*	*Response*	
67	F	Centrocytic	IV	3	–	–	–	–	–	PR	
39	M	CC-CB	IV	0	–	–	–	–	–	PR	
64	F	Lymphocytic	IV	2	–	–	–	–	–	PR	
67	F	CC-CB	IV	1	–	–	–	–	–	PR	
48	F	CC-CB	III	0	SC	–	–	–	16	PR	
51	M	Lymphocytic	IV	0	SC	–	–	–	12	PR	
50	F	CC-CB	IV	1	CC	Rt	–	–	24	PR	
62	M	CC-CB	III	0	CC	Rt	–	–	10	PR	
37	F	CC-CB	IV	0	CC	Inf	–	–	36	PR	
58	F	CC-CB	III	0	CC	Inf	–	–	48	PR	
48	F	CC-CB	III	0	CC	SC	–	–	60	PR	
44	F	CC-CB	IV	2	CC	Inf	–	–	6	PR	
40	M	CC-CB	IV	1	SC	Inf	–	–	0,5	PR	
70	M	Lymphocytic	IV	1	Inf	–	–	–	0,5	PR	
51	F	Centrocytic	IV	2	SC	CC	–	–	1,5		SD
66	M	Lymphocytic	IV	1	SC	–	–	–	0,5		SD
66	M	CC-CB	IV	1	CC	CC	–	–	1,5		SD
54	M	Centrocytic	IV	2	CC	HC	–	–	1		SD
62	M	Centrocytic	IV	1	CC	–	–	–	0,5		Prog
68	M	CC-CB	IV	3	CC	Rt	CC	–	1		Prog

Abbreviations: PT, previous therapies, from left to right in temporal succession; SC, single agent chemotherapy; CC, combination chemotherapy; HC, high dose intensive chemotherapy; Rt, radiotherapy; Inf, interferon; CC-CB, centroblastic-centrocytic; PR, partial response; SD, stable disease; Prog, progression.

Seventy percent of the patients (14 of 20; 95% CI, 50 to 90%) had a partial response; 20% (4 of 20; 95% CI, 2.5% lo 37.5%) had stable disease and 10% (2 of 20) progressed on therapy. The response after 1 month in patients subdivided according to the previously received therapies is described in Table 2.

Table 2. Response to regimen after 1 month; patients subdivided according to the previously received therapies

NHL	Number of patients	Response (number of patients) PR	SD	Prog
Previously untreated patients	4	4	–	–
Previously treated patients				
Patients with TFT≥6 months in first relapse	8	8	–	–
Patients with TFT≤1.5 months non-responding or progressed during interferon therapy	2	2	–	–
Patients with TFT≤1.5 months non-responding or progressed during single agent or combination chemotherapy	6		4	2
All the patients	20	14	4	2

Abbreviations: PR, partial response; SD, stable disease; Prog, progression.

One hundred percent (4 of 4) of the previously untreated patients, 100% (8 of 8) of the patients wilh TFT ≥ 6 months and 100% of the patients with TFT ≤ 1.5 months non-responding or progressed during interferon therapy, had a partial response. Among the patients with TFT ≤ 15

months non-responding or progressed during single agent or combination chemotherapy, 66.6% (4 of 6; 95% CI, 28% to 104%) had stable disease and 33.3% (2 of 6) progressed. The response to the treatment prosecution is analitically described in Table 3.

Table 3. Response to the treatment prosecution

Age	Sex	Histology	Response	TTP (months)	Follow-up (months)
67	F	Centrocytic	SD	–	25
39	M	CC-CB	CR	–	21
64	F	Lymphocytic	CR	–	21
67	F	CC-CB	SD	–	7
48	F	CC-CB	SD	–	21
51	M	Lymphocytic	SD	–	21
50	F	CC-CB	CR	–	21
62	M	CC-CB	CR	–	21
37	F	CC-CB	SD	–	9
58	F	CC-CB	SD	–	22
48	F	CC-CB	CR	–	19
44	F	CC-CB	CR	–	18
40	M	CC-CB	CR	–	22
70	M	Lymphocytic		–	19
51	F	Centrocytic	PR	–	15
66	M	Lymphocytic	Prog	21	–
66	M	CC-CB	Prog	15	–
54	M	Centrocytic	Prog	7	–

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; Prog, progression; TTP, time-to-progression.

Among the 14 patients that after 1 month had partial response, there was no disease progression (average follow-up time of 21 months, range, 7 to 25), and 50% of these patients (7 of 14; 95% CI, 24% to 76%) obtained a complete response. Among the 4 patients that after 1 month had stable disease, 25% (1 of 4) had a partial response and 75% (3 of 4) progressed (mean time to progression 14.3 months, range, 7 to 21). The response to the treatment prosecution in patients subdivided according to the previously received therapies is described in Table 4.

Table 4. Response to the treatment prosecution; patients subdivided according to the previously received therapies

NHL	Number of patients	Response (number of patients) CR	PR	SD	Prog
Previously untreated patients	4	2	–	2	–
Previously treated patients					
Patients with TFT≥6 months in first relapse	8	4	–	4	–
Patients with TFT≤1.5 months non-responding or progressed during interferon therapy	2	1	–	1	–
Patients with TFT≤1.5 months non-responding or progressed during single agent or combination chemotherapy	4	–	1	-	3
All the patients	18	7	1	7	3

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; Prog, progression.

All the 20 patients were evaluable for the toxicity. The must common side effects were gastrointestinal signs. Twenty-five percent of the cases had diarrhea first grade (2 patients) or second grade (3 patients); 20% (4 patients) had nausea or grade 1 vomit, and 5 patients had loss of appetite and anorexia. These side effects did not require suspension of the therapy but only all adjustment of the dose of somatostatin. Drowsiness was observed in 20% (4 of 20) of patients, and required an adjustment of the daily schedule of administration of melatonin (20 mg/day were subdivided into three doses, instead of two, one of which at bedtime).
Twenty five percent of patients (5 of 20) had grade I hyperglycemia (≤ 160/dL) and 20% (4 of 20) showed ankle and/or face edema; in both these cases the dose of ACTH was reduced to 0.5 mg/week.

DISCUSSION

The low-grade NHL at advanced stage are stili incurable disease, whose treatment, just in consequence of these therapeutic limits, is very disputed, coexisting single agent chemotherapy, combination chemotherapy, combined radiotherapy- chemotherapy, high-dose intensive chemotherapy with autologous bone marrow transplanation or autologous blood progenitor cell transplantation.

In this study we evaluated toxicity and efficacy of a regimen, known as Di Bella's multitherapy (DBM), resulting by the association of a chemotherapeutic agent, the cyclophosphamide, with other non-myelosuppressive substances (somatostatin, bromocriptin, retinoids, melatonin and ACTH).

The results we obtained - seventy percent of partial responses after 1 month, fifty percent of which became complete responses going on with the treatment - have been really better than the ones described with single agent chemiotherapy with alkylants, with wiich Kimby et al described 36% of global response with complete response in 5% of 132 previously untreated patients, [38] or than the ones described by Witzig et al with somatostatin as single agent (36% of partial) response in 28 previously treated and untreated patients). This demonstrates the therapeutic superiority of such a pharmacological association versus its single costituents.

Moreover, the activity of the regimen depended on the kind of previous therapy and on the TFT. We documented 100% of global response among the previously untreated patients, the patients in first relapse with TFT ≥ 6 months and the patients with TFT ≤ 1.5 months non-responding or progressed during interferon therapy.

This result, better than others obtained with widely used chemotherapy regimens [Kimby et al described 60% of global response with CHOP in 127 previously untreated patients with lowgrade NHL stage III and IV] [38], and the very good tolerance of DBM (all the patients carried on the treatment at home, going on with their normal activities) suggest further clinical trials using this regimen in NHL

With regard to this, the recent availability of depot formulations of somatostatin may allow a better feasibility of this regimen, where the main discomfort was in the daily SQ injection of somatostatin, especially if done with syringe pump.

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