Archives of Disease in Childhood, 1987, **62**, 1102–1106

Cyclophosphamide treatment of steroid dependent nephrotic syndrome: comparison of eight week with 12 week course

REPORT OF ARBEITSGEMEINSCHAFT FÜR PÄDIATRISCHE NEPHROLOGIE*
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SUMMARY In a prospective study (Cytotoxic Drug Study II), 18 children with steroid dependent nephrotic syndrome and steroid toxicity were treated with cyclophosphamide (2 mg/kg body weight/day) for 12 weeks in combination with reducing doses of prednisone (group A). This group was compared retrospectively with 18 children with steroid dependent nephrotic syndrome, studied as part of the Cytotoxic Drug Study I, and who had received cyclophosphamide for eight weeks (group B). There were no differences between the groups in age at the onset of the nephrotic syndrome, age at entry into the study, and duration of the nephrotic syndrome before entry into the study. The number of relapses during the six months before the treatment was the same in both groups. Two years after treatment 12 of 18 children treated with cyclophosphamide for 12 weeks were still in remission. By contrast, only four of 18 children treated with cyclophosphamide for eight weeks were still in remission. The cumulative rates of sustained remissions were significantly higher (67% and 22%, respectively) in group A. All relapses were observed within 400 days of stopping cytotoxic treatment. No severe side effects of cyclophosphamide occurred up to two years after treatment had been stopped. We conclude that for children with steroid dependent nephrotic syndrome and steroid toxicity cyclophosphamide treatment should be prolonged to 12 weeks to increase the likelihood of a prolonged remission.

In a previous study it was shown that 78% of children with steroid dependent nephrotic syndrome who were treated with cyclophosphamide for eight weeks relapsed soon after the treatment was stopped.[1] The question was raised therefore whether the unfavourable result in this group was due to a primary resistance to cytotoxic drugs or to the low cumulative dose of cyclophosphamide used (112 mg/kg body weight). The reported experience of other groups was controversial,[2–6] and did not answer the question because the authors did not distinguish between patients with and without steroid dependence who relapsed frequently.

The Arbeitsgemeinschaft für Pädiatrische Nephrologie therefore started another prospective multicentre study in which children with steroid dependent nephrotic syndrome and steroid toxicity were treated with cyclophosphamide for 12 weeks (group A) instead of eight weeks as in the first trial (group B). This study (Cytotoxic Drug Study II) aimed to investigate the effect of a 12 week course of cyclophosphamide on the duration of remission and on the rates of relapse in children with steroid dependent nephrotic syndrome with minimal histological changes, and to compare the results of the 12 week course with those of the eight week course in the same type of patients studied in the first trial.[1]

Patients and Methods

We studied children aged 2 to 16 years with nephrotic syndrome with minimal histological changes who responded to treatment with prednisone with complete remission, but who subsequently relapse frequently, developing steroid dependence and becoming toxic to steroids due to the prolonged

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treatment with prednisone. The definitions and criteria for nephrotic syndrome, remission, and relapse were the same as those used by the International Study of Kidney Disease in Children[7] and by the Arbeitsgemeinschaft für Pädiatrische Nephrologie.[8] Patients with steroid dependent nephrotic syndrome were defined as: those in whom two consecutive relapses occurred during the alternate day prednisone treatment regimen given for an earlier relapse; or within 14 days after the end of an alternate day prednisone regimen ('fast' relapse); or in whom two of four relapses in a period of six months were fast relapses.[1] Renal biopsies were performed in each patient before starting the cytotoxic treatment. Patients previously treated with any cytotoxic drug were excluded from the study.

For steroid treatment only a standard initial and relapse regimen of prednisone was used.[1] The first attack was treated with 60 mg/m^2/day given in three divided doses (total dose not more than 80 mg per day) for four weeks, followed by prednisone on alternate days with 40 mg/m^2/48 hours given as a single dose on the morning of every other day for four weeks. Relapses were treated with 60 mg of prednisone/m^2/day until the urine had been free of protein for three days; patients were then given four weeks of treatment on alternate days at a dose of 40 mg/m^2.

For cytotoxic treatment only cyclophosphamide at a dose of 2 mg/kg was used. In the first Cytotoxic Drug Study (CYTO I), which ran from 1977 to 1981, cyclophosphamide was given for eight weeks (total dose 112 mg/kg). Patients who were treated with chlorambucil in that study were not included in the present comparison. In the second Cytotoxic Drug Study (CYTO II), which ran from 1981 to 1986, cyclophosphamide was given for 12 weeks (total dose 168 mg/kg). The total cumulative doses remained below the accepted levels for gonadal toxicity.[9 10]

Statistical analysis was performed by Student's *t* test, the χ^2 test, and the Lee-Desu test for life table analysis[11] using the Statistical Package for the Social Sciences-X (SPSS-X) statistical software program.

PROTOCOL

Patients with steroid dependent nephrotic syndrome who fulfilled the above criteria were treated with cyclophosphamide according to the treatment protocol shown in table 1. Informed parental consent was obtained for each patient. All patients were admitted to hospital for the first few days of the study. The cyclophosphamide was started when the proteinuria which had indicated the last relapse had been absent for three days after standard prednisone treatment for the relapse. Reducing doses of prednisone were given (table 1). To maintain the protective effect of prednisone on leucopenia induced by cyclophosphamide, the prednisone treatment was also prolonged to 12 weeks in CYTO II. All medication thereafter was discontinued.

The patients were followed up closely in the renal outpatient clinics of the participating hospitals. Proteinuria was measured daily with a strip test (Albustix) by the patient's parents. If an acute complication occurred during treatment (especially leucopenia or thrombocytopenia) cyclophosphamide was discontinued until the patient had recovered. At the end of the treatment the missed period of cyclophosphamide treatment was made up.

The effectiveness of treatment was judged by the duration of remission and the number of relapses after the full course of treatment.

Results

A total of 18 patients entered the CYTO II; seven

Table 1 *Protocols of Groups A and B*

Group A (1981–1986)	*Group B (1977–1981)*
1 Steroid responsive nephrotic syndrome with minimal histological change, steroid dependency, and steroid toxicity	
2 Relapse	
3 Prednisone (60 mg/m^2 of body surface area/day) until urine free from protein for three days	
Cyclophosphamide:	*Cyclophosphamide*:
2 mg/kg/day×12 weeks	2 mg/kg/day×8 weeks
Total dose=168 mg/kg	Total dose=112 mg/kg
Prednisone:	*Prednisone*:
60 mg/m^2/48h×4 weeks	60 mg/m^2/48h×4 weeks
40 mg/m^2/48h×1 week	40 mg/m^2/48h×1 week
30 mg/m^2/48h×1 week	30 mg/m^2/48h×1 week
20 mg/m^2/48h×1 week	20 mg/m^2/48h×1 week
10 mg/m^2/48h×5 weeks	10 mg/m^2/48h×1 week
Total dose = 1340 mg/m^2	Total dose = 1200 mg/m^2

Table 2 *Details of patients studied*

	Group A	*Group B*
No of patients	18	18
Male:female	7:11	9:9
Mean (SD) age when nephrotic syndrome diagnosed	5·8 (4·0)	4·9 (2·7)
Mean (SD) age at entry to study	8·6 (4·0)	6·9 (2·9)
Mean (SD) duration of nephrotic syndrome (years)	2·9 (2·5)	1·9 (1·5)
Mean (SD) no of relapses in the past 6 months	4·4 (1·0)	4·8 (1·2)
Mean (SD) no of fast relapses	3·3 (1·7)	3·5 (1·8)
Mean (SD) cumulative dose of prednisone in the past 6 months (mg/kg/patient)	164·7 (56)	158·6 (84)

girls and 11 boys were treated with cyclophosphamide for 12 weeks (group A). These were compared with 18 patients (nine boys and nine girls) who had been treated with cyclophosphamide for eight weeks in CYTO I (group B) (table 2). The age at onset of nephrotic syndrome, the age at entry into the study, and the duration of nephrotic syndrome before entry into the study were comparable in both groups.

The mean number of relapses in the six months before cyclophosphamide treatment was 4·4 in the group A, and 4·8 in group B. Most of them were 'fast relapses'[1]—that is, 3·3 fast relapses patient/6 months in group A and 3·5 in group B. All the patients therefore fullfilled the criteria for steroid dependent nephrotic syndrome.

The cumulative dose of prednisone administered within six months of entry into the study was the same in both groups, 158·6 and 164·7 mg/kg/patient, respectively. Toxic side effects of prolonged steroid treatment were seen in all patients; these included osteoporosis, cataracts, severe obesity, and psychic disturbances.

All patients were followed up for at least two years. In group A 12 patients (67%) remained in complete remission without any relapse for two years, in contrast, only four patients in group B (22%) remained in remission (table 3). The difference was highly significant (p=0·018).

Using the life table analysis method of Cutler and Ederer the time sequence of relapses in both groups was analysed (fig 1).[11] The difference at two years was again significant (p=0·018, Lee-Desu test).

All relapses after cyclophosphamide treatment occurred within 400 days after the end of the treatment (fig 2). Only the patients who relapsed are shown: 14 patients treated in group B and six patients in group A. There are no differences in the time sequence of relapses.

No severe side effects of cyclophosphamide (haemorrhagic cystitis, severe infections, thrombocytopenia, or neoplastic disease) occurred during either the treatment or the following two years. The only mild and transient side effect was leucopenia (white cell count $<3\times10^9$/l). This occurred in two patients during the eighth week of the cytotoxic treatment and lead to an interruption of cyclophosphamide treatment for 11 days.

Table 3 *No of patients with or without relapse after 12 weeks (group A) or eight weeks (group B) of cyclophosphamide treatment*

	Group A No (%)	*Group B No (%)*	*p* Value*
Patients with relapse	6 (33)	14 (78)	
Patients without relapse two years after treatment	12 (67)	4 (22)	0·018
Total patients	18 (100)	18 (100)	

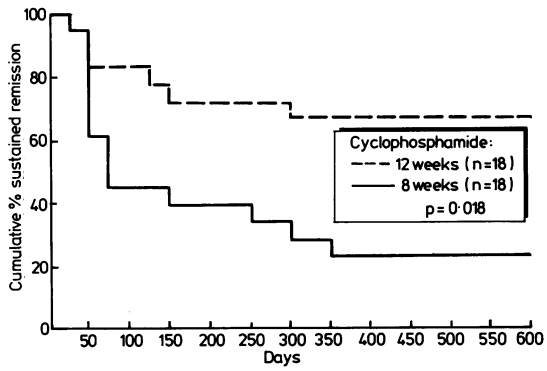

Fig 1 *Life table analysis of cumulative rate of sustained remission: comparison of eight week with 12 week treatment with cyclophosphamide after two years.*

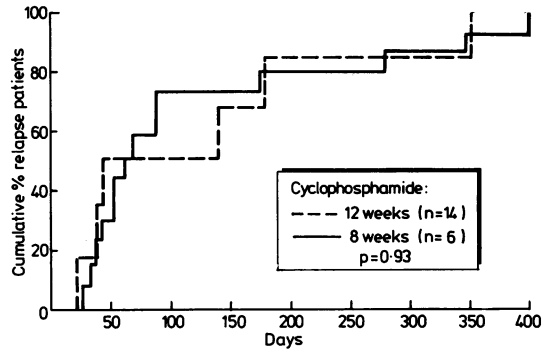

Fig 2 *Life table analysis of time sequence of relapses in groups A and B.*

Discussion

The results of the present study confirm the importance of distinguishing between two forms of frequent relapsing nephrotic syndrome, one with and the other without steroid dependency. CYTO I showed that patients who relapsed frequently but were not steroid dependent benefited from a course of cytotoxic drugs lasting eight weeks, either with cyclophosphamide or chlorambucil,[1] 72% of them remaining in prolonged remission. In contrast, those who relapsed frequently and were steroid dependent did not benefit from an eight weeks' course of cytotoxic drugs as 72% of them relapsed again soon after treatment was discontinued. A similar result

was obtained by Garin *et al* in a retrospective study.[12] Some other authors have shown that higher cumulative doses of cyclophosphamide may result in longer lasting remissions.[5 13 14] Unfortunately, none of these studies reported separate evaluations of steroid dependent and non-steroid dependent patients.

Because the results of an eight week course with cyclophosphamide in steroid dependent nephrotic syndrome had already been obtained in the CYTO I study, we did not consider it ethical to repeat this part of the trial for a randomised study. We therefore studied a single group having a 12 week course of treatment, and compared the results with those of the group in the CYTO I study. This was not a strictly randomised study; the groups were, however, comparable except for the time of the study.

For group A we preferred to use cyclophosphamide alone and not chlorambucil which had been used in the previous study because there is ample evidence that the chlorambucil might produce permanent toxic damage to the male gonads in a cumulative dose over 8 mg/kg.[15 16] The cumulative dose for cyclophosphamide in this study was 168 mg/kg—that is, below 200 mg/kg, the safe borderline for gonadal toxicity.[9 10] Patients from our first cytotoxic trial were assessed five years after an eight week course of cyclophosphamide or chlorambucil and no abnormalities in gonadal endocrine function were found.[17] Feehally *et al*[18] could not detect any abnormality of cellular immune state one year after treatment of nephrotic syndrome with minimal histological changes with 2·5 mg/kg cyclophosphamide for eight weeks (cumulative dose 140 mg/kg). Nevertheless, long term side effects (such as malignant transformation) in children are still not known. Acute side effects of treatment were rare; occasionally a mild and transient leucopenia occurred which led to a short interruption in treatment. Serious side effects were not encountered.

The results of the present study give a clear answer to the question of effectiveness: in most of the children with steroid dependent nephrotic syndrome studied (67%), a prolonged remission was achieved after a 12 week course of cyclophosphamide; only 22% of those given an eight week course remained in prolonged remission.

Although the total dose of prednisone given simultaneously with cyclophosphamide was slightly higher in group A (1340 mg/m^2 in group A and 1200 mg/m^2 in group B), it is unlikely that this could be responsible for the difference. In an earlier study we showed that the amount and mode of administration of the prednisone had no lasting effect on the rate of relapse in patients who relapsed frequently.[19]

There was no difference in the time sequence of relapses between the two groups (fig 2). All relapses occurred within 400 days of stopping treatment. Although there is always a possibility of a late relapse—that is, after two years—our findings indicate that the effects of the treatment could be judged after 400 days.

We conclude that patients with the frequently relapsing nephrotic syndrome should be treated differently depending on whether their disease is steroid dependent or not steroid dependent. Using this criterion it is possible to give the lowest effective dose of the cytotoxic drug when they do become steroid toxic. Patients who relapse frequently but who are not steroid dependent should be treated with cyclophosphamide (2 mg/kg/day) for eight weeks and, those who relapse frequently but are steroid dependent should be given cyclophosphamide for 12 weeks.

This study was supported by grants EZ 1–34844 of VW Foundation, Hannover.

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Received 15 May 1987

Fifty years ago

Familial progressive diffuse cerebral sclerosis of infants

Dorothy S Russell and K H Tallerman (London)—Arch Dis Child 1937;**12**:71–86

Two siblings, one boy and one girl, of Jewish parents who were first cousins, died of similar illnesses. The onset in the early weeks of life was heralded by reluctance or inability to suck and swallow. Convulsive attacks were followed by head retraction, absence of visual recognition, rigidity, and exaggerated reflexes and extensor plantar responses. Ophthalmoscopy showed no gross abnormality and no cherry red spot. Postmortem examination, obtained only in the second child, revealed gross abnormalities in the central nervous system of a degenerative nature, involving destruction in the cerebral and cerebellar cortices. Associated with this was infiltration with amoeboid forms of microglia containing lipoid material, which gave a positive reaction with Herxheimer's and Marchi's methods. Numerous astrocytes were often fibrillated with large cell bodies and clear, glassy cytoplasm. Extensive destruction of myelin was shown by ballooning of the sheaths and by wide dissemination of globular fragments of myelin. Cellular infiltrations of the type seen in infections of the central nervous system were absent.

In discussing the diagnosis the authors pointed out that similar clinical features could be seen in a variety of different encephalopathies. Tay-Sachs disease was excluded by the absence of the characteristic macular changes. The heavy involvement of the cortex shown after death was taken to exclude Schilder's disease and Pelizaeus-Merzbacher disease. In the absence of any real knowledge of the aetiology of the different types of diffuse cerebral sclerosis in young children these two cases were considered to fall into the group earlier described by Krabbe as a familial infantile form of "diffuse brain sclerosis".

Comment. Krabbe's globoid body leucodystrophy is now recognised as one of the lipid storage diseases affecting the central nervous system. Inherited as an autosomal recessive, deficiency of galactocerebroside β galactosidase can be shown by specific enzyme assay in leucocytes or cultured fibroblasts. Tests for heterozygotes are available.
(Dorothy Russell was a distinguished neuropathologist. In 1949 she produced a most valuable contribution on the pathology of hydrocephalus for the special report series of the Medical Research Council. I first met Kenneth Tallerman at meetings of the British Paediatric Association at Windermere before the second world war when he offered a friendly welcome to a very young Scottish paediatrician. He was President of the BPA in 1958, the last year that its annual general meeting was held at Windermere.)

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