Comparative trial of 2 weeks and 8 weeks cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood

T. M. BARRATT, J. S. CAMERON, C. CHANTLER, C. S. OGG, and
J. F. SOOTHILL

From the Department of Immunology, Institute of Child Health; and the Department of Paediatrics and Department of Medicine, Guy's Hospital, London

Barratt, T. M., Cameron, J. S., Chantler, C., Ogg, C. S., and Soothill, J. F. (1973). *Archives of Disease in Childhood*, 48, 286. **Comparative trial of 2 weeks and 8 weeks cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood.** A comparative trial of 2 weeks and 8 weeks cyclophosphamide therapy given during steroid-maintained remission in 32 children with steroid-sensitive relapsing nephrotic syndrome of childhood is reported. A significantly greater number of the 16 children who received 2 weeks therapy relapsed after steroid withdrawal than of those who received 8 weeks therapy. Relapses occurred later after 2 weeks cyclophosphamide therapy than in our previous experience of steroid treatment alone.

We have previously shown (Barratt and Soothill, 1970) that the administration of an 8-week course of cyclophosphamide in a dosage of 3 mg/kg per day during a steroid-maintained remission to children with steroid-sensitive frequent-relapsing nephrotic syndrome confers substantial protection from relapse after subsequent steroid withdrawal. Though overt toxicity during the 8-week treatment course was minor, we observed that 'There is no evidence that the present dose (i.e. 3 mg/kg per day for 8 weeks) is the lowest effective one. In view of the concern about the use in young children of a drug which interacts with genetic material, we feel that there is an obligation to use as little as possible'.

This concern has been heightened by the recent report (Fairley, Barrie, and Johnson, 1972) that adult patients treated with cyclophosphamide develop persistent azoospermia within a few weeks of starting the drug. It is necessary, therefore, to determine whether a similar stability of remission of nephrotic syndrome can be induced by shorter courses of cyclophosphamide. In this paper we report a comparative trial of 2 weeks and 8 weeks cyclophosphamide therapy of steroid-sensitive frequent-relapsing nephrotic syndrome of childhood.

Methods, definitions, and trial design

Methods, definitions, and patient selection were all as previously described (Barratt and Soothill, 1970).

Treatment regimens. Children with steroid-sensitive frequent-relapsing nephrotic syndrome were entered into the trial in a stable state of remission on their maintenance prednisolone dosage, and were randomly allocated to one of two regimens, A' or B' (Fig. 1). The weeks are numbered from the start of prednisolone withdrawal and the week of entry is therefore -8. In both regimens maintenance prednisolone was continued from week -8 to -1, and then withdrawn in a standard, approximately logarithmic manner during weeks 1 to 8.

FIG. 1.—Treatment schedules.

Cyclophosphamide was administered in a dosage of 3 mg/kg per day (mean weight for children of same height) during weeks -2 and -1 in regimen A', and from week -8 to -1 in regimen B'. Check for haematological toxicity was as previously described (Barratt and Soothill, 1970); in no case was the cyclophosphamide course interrupted.

Determination of relapse. All patients were treated at home; the parents were instructed to test the urine daily with Albustix and to report to hospital if a 2+ reaction was observed for 2 days. Two urine specimens were then obtained for estimation of albumin/creatinine concentration ratio (A_u/C_u). In addition, random urine samples for A_u/C_u were collected in weeks 8, 24, and 60. A relapse was defined as A_u/C_u greater than 1.0. The critical observation in each child, designated 'relapse' or 'no relapse', was whether or not relapse criteria had been satisfied in or before week 24.

Statistical design. 32 children were entered into the trial. A system of restricted random allocation

was achieved by withdrawal of sealed cards from a box so that equal numbers were allocated to each regimen after 16 and 32 cases had been entered. The distribution of relapses in or before week 24 was analysed by the exact test (2×2 contingency tables: Finney *et al.*, 1963) on two occasions, after 16 and 32 cases reached week 24. The null hypothesis was that there were not more relapses in group A' than in group B': a predetermined significance level for a one-sided trial was set at 5% ($\alpha = 0.05$), and the technique of Armitage, McPherson, and Rowe (1969) for assessing repeated significance tests on accumulating data was applied, such that at each of the two analyses $\alpha = 0.030$. (This result is strictly appropriate for normally distributed variables, but is likely to be approximately true for tests on 2×2 tables.)

Departures from protocol. 3 children were allocated to 2 weeks of cyclophosphamide (regimen A') but relapsed in weeks -8 to -3, i.e. after allocation but before cyclophosphamide therapy. These patients were assumed to have been assigned the wrong maintenance dose of prednisolone, and were withdrawn

TABLE I
Results

Patient	Age at entry to trial (yr)	Duration from first episode to entry (yr)	Height centile	Maintenance prednisolone (mg/kg per day)	Duration of follow-up in trial (wk)	Week of relapse
<i>Group A'</i>						
1	9.7	7.7	50	0.33	103	84
2	5.9	1.7	3	0.49	97	—
3	6.6	2.1	50	0.22	85	11
4	3.9	1.8	90	0.22	84	31
5	10.5	7.2	<3	0.32	76	10
6	9.4	5.2	25	0.23	75	44
7	13.6	9.1	10	0.21	63	29
8	13.8	4.1	3	0.25	57	—
9	6.6	3.1	50	0.36	57	12
10	8.5	2.9	25	0.34	57	10
11	3.7	1.1	50	0.33	52	24
12	3.7	1.1	25	0.66	44	6
13	6.2	3.4	25	0.46	41	—
14	3.5	0.7	10	0.45	31	20
15	2.8	0.8	75	0.56	24	2
16	10.0	4.8	25	0.23	20	4
<i>Group B'</i>						
1	4.5	3.5	75	0.49	106	—
2	5.1	3.0	<3	0.56	102	22
3	4.4	1.3	3	0.36	100	—
4	4.3	2.8	3	0.88	92	25
5	10.4	2.6	50	0.13	84	—
6	7.7	4.8	3	0.46	74	—
7	6.7	5.0	<3	0.32	65	—
8	12.9	9.9	10	0.19	64	—
9	14.6	9.0	25	0.14	63	—
10	7.7	3.9	25	0.30	57	—
11	8.9	4.1	75	0.14	56	—
12	11.7	4.4	90	0.13	41	—
13	12.4	6.9	3	0.32	38	—
14	8.4	2.7	50	0.32	28	—
15	6.1	4.7	3	0.44	27	—
16	4.1	0.8	75	0.48	24	—

Note: Group A': prednisolone (wk -8 to -1), withdrawal (wk 1 to 8); cyclophosphamide (wk -2 and -1).
Group B': prednisolone (wk -8 to -1), withdrawal (wk 1 to 8); cyclophosphamide (wk -8 to -1).

FIG. 2.—Trial results.

from the trial. Any bias from this is likely to operate in favour of regimen A' by withdrawal of relapse-prone patients from that group. Another child was withdrawn from group A' because the cyclophosphamide was inadvertently administered at the wrong time. One child (A' 14) developed proteinuria and oedema in week 20 but A_u/C_u was not estimated; in analysis of the trial he has been classified as relapse.

Results

Details of the children entered into the trial are given in Table I. The two groups did not differ significantly by Rank-sum test in median age of entry into the trial, duration of illness, height centile, or maintenance prednisolone dosage. Serum β_1 C-globulin was within the normal range, and creatinine clearance exceeded 80 ml/min per 1.73 m^2 surface area in all patients. Selectivity of proteinuria was assessed in 14 children: 13 had highly selective proteinuria (IgG/albumin clearance ratio $<20\%$), but in B'4 the proteinuria was less

selective (34%). Renal biopsy was not undertaken routinely, but had been done in 9 children (not including B'4): in none were histological abnormalities of the glomeruli observed by light microscopy.

Table I and Fig. 2 indicate the duration of follow-up in the trial and the week in which relapse was observed. At the first analysis, 2 of 8 children in group A' had relapsed, in contrast to 1 of 8 children in group B', an insignificant difference. However, after 32 patients completed the trial, with 9 of the 16 children in Group A' relapsing in contrast to one in group B', the difference was significant ($P = 0.003$ by the exact test, well beyond the critical level of 0.03 appropriate for correction for multiple access to trial data) (Table II). Thus 2 weeks of cyclophosphamide therapy is less effective in the group as a whole than 8 weeks in the prevention of relapse of steroid-sensitive nephrotic syndrome of childhood.

TABLE II
Distribution of relapses

No. of patients	Week 24			Week 60		
	A' (2 weeks CP)	B' (8 weeks CP)	P*	A' (2 weeks CP)	B' (8 weeks CP)	P*
16	2/8	1/8	>0.05	5/8	2/8	>0.05
32	9/16	1/16	0.003			

*Probability, not corrected for multiple access to trial.
CP, cyclophosphamide.

Discussion

The data presented establish that an 8-week course of cyclophosphamide results in a more stable remission of steroid-sensitive frequent-relapsing nephrotic syndrome than does a 2-week course. This result confirms our previous conclusion (Barratt and Soothill, 1970) that 'cyclophosphamide, administered during a steroid-induced remission, influences the probability of relapse upon subsequent withdrawal of steroids'. In the present trial the administration of prednisolone was exactly matched in the two treatment regimens, which overcomes the acknowledged imperfection of the earlier trial design in which the cyclophosphamide-treated group also had a longer period of maintenance prednisolone therapy.

The cases were randomly allocated to one of the two treatment groups, which did not differ significantly in the median values of the descriptive variables listed in Table I, in β_2 -globulin, or in creatinine clearance. Renal biopsy was undertaken in only 9 children and showed no histological abnormality of the glomeruli on light microscopy. There were no significant differences between the median values of the variable listed above in the patients in the present trial and in those in the previous trial, who were selected by the same criteria. As 28 of the 30 patients in the previous trial were shown to have minimal histological abnormalities, it is safe to assume a similar histological pattern in the present series, with the possible exception of patient B'4, who had surprisingly poorly selective proteinuria.

The design of the present trial led to one difficulty, in that children allocated to 2 weeks cyclophosphamide who had been assigned an incorrect maintenance prednisolone dosage tended to relapse after entry but before cyclophosphamide therapy. 3 such patients had to be withdrawn from the trial after allocation, which results in some inequality between the treatment groups. However, as the effect is to remove unstable patients from the trial, the bias operates in favour of the

2-week group, and therefore does not jeopardize our conclusion that the 2-week course was less effective.

Of the second group of 8 patients, 7 who were admitted to the 2-week regimen relapsed in or before week 24 in contrast to only 2 in the first group ($0.05 > P > 0.02$; exact test; Finney *et al.*, 1963). The reason for this difference is not apparent.

It is interesting to compare the data presented in this trial and the earlier one (Barratt and Soothill, 1970). The two 8-week cyclophosphamide courses were exactly similar in design and gave similar results. The incidence of relapse in both groups has, therefore, been grouped for the 'survivorship analysis' (Armitage, 1971) presented in Fig. 3. The percentage remaining in remission declines apparently exponentially; but by 1 year only 25% have relapsed. If relapse represents a response to

FIG. 3.—'Survivorship analysis' (Armitage, 1971) after different durations of cyclophosphamide therapy. There is apparently an exponential relapse. The straight lines were drawn by eye.

a trigger after recovery from sustained immunosuppression, an increasing rate of relapse might have been expected. The finding of a constant proportionate relapse rate suggests a fixed state of altered reactivity with relapse depending on the occurrence of a more powerful external stimulus than is required in untreated patients.

There is some suggestion that the rate of relapse is higher in the group who received no cyclophosphamide than in the 2-week group: their median time to relapse was 13 weeks in contrast to 22 weeks with the latter regimen ($P = 0.06$; Rank-sum test; Armitage, 1971). However, other factors such as differing prednisolone schedules and lack of random allocation limit the validity of conclusions drawn from such a comparison. It would be important to know whether a larger proportion of patients enter permanent remission after 2 weeks of cyclophosphamide than if no cyclophosphamide was given. Such an effect, if present, must be small, and if our 1-year data are representative, requires a trial size of at least 100 patients for statistical confirmation.

In view of the anxieties inherent in the use of cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood, however, it may still be rational to treat children in whom corticosteroid therapy is unsatisfactory with 2 weeks of

cyclophosphamide before resorting to an 8-week course, but the proportion of children likely to benefit from such treatment is small.

We thank the paediatricians who referred us cases for entry into the trial and assisted in their management, Drs. R. J. K. Brown, P. J. N. Cox, R. H. Dobbs, R. C. Evans, A. W. Fergusson, G. Katz, S. J. R. Macoun, A. P. Mowat, A. T. Piesowicz, H. A. J. Reay, T. S. Rodgers, M. E. Smith, M. E. R. Stoneman, C. H. C. Upjohn, P. G. Wallis, P. E. Walker, and M. J. Wilmers.

We also thank Professor P. Armitage for statistical advice and Dr. J. M. Simister of Ward Blenkinsop Ltd. for generous financial assistance.

REFERENCES

- Armitage, P. (1971). *Statistical Methods in Medical Research*. Blackwell, Oxford.
- Armitage, P., McPherson, C. K., and Rowe, B. C. (1969). Repeated significance tests on accumulating data. *Journal of the Royal Statistical Society, Series A (General)*, **132**, 235.
- Barratt, T. M., and Soothill, J. F. (1970). Controlled trial of cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood. *Lancet*, **2**, 479.
- Fairley, K. F., Barrie, J., and Johnson, W. (1972). Sterility and testicular atrophy related to cyclophosphamide therapy. *Lancet*, **1**, 568.
- Finney, D. J., Latscha, R., Bennett, B. M., and Hsu, P. (1963). *Tables for Testing Significance in a 2×2 Contingency Table*. Cambridge University Press, London.

Correspondence to Dr. T. M. Barratt, Department of Immunology, Institute of Child Health, 30 Guilford Street, London W.C.1.