SISTER CHROMATID EXCHANGES IN CHILDREN TREATED WITH AZATHIOPRINE AND CYCLOPHOSPHAMIDE, AND EFFECT OF PROTEIN-BOUND POLYSACCHARIDE (PS-K)

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Summary Sister chromatid exchange (SCE) was examined in cultured lymphocytes of nephrotic children and some other juvenile patients treated with immunosuppressive agents, azathioprine (AZA) and cyclophosphamide (CYC), and CYC in combination with a protein-bound polysaccharide (PS-K). The frequency of SCEs in children treated with CYC showed a highly significant increase as compared with the non-treated control. AZA treatment also resulted in an increased frequency of SCEs, though the mean value was lower than that obtained for CYC. An extremely high frequency of SCEs resulted from a combined treatment with AZA and CYC. After 3–4 months of cessation of CYC medication, the frequency of SCEs returned to the normal level. The SCE frequency decreased significantly when CYC was used in combination with PS-K.

INTRODUCTION

Cyclophosphamide (CYC) and azathioprine (AZA) have been used clinically as immunosuppressive agents for the treatment of certain nephrotic syndromes and immunologic diseases of children, notwithstanding their mutagenic and teratogenic potential. But it may be the present state that the agents are used reluctantly because of few effective drugs to these diseases. In our clinic, for the purpose of reducing the several side-effects of CYC for the treatment of nephrotic children, a protein-bound polysaccharide, PS-K, isolated from *Basidiomycetes* (Tsukagoshi, 1974), has been successfully used.

The purpose of the present investigation is to study the cytogenetic effect, as revealed by the SCE test, of immunosuppressive agents, AZA and CYC, and of CYC combined with PS-K.

MATERIALS AND METHODS

The non-treated control group included 20 normal healthy children (10 males, 10 females, age: 6–15-years-old), who had no history of medication, X-ray exposures, or any significant viral disease, at least for the last six months. As indicated in Table 1, the same number of treated-children were examined (9 males, 11 females, age: 5–14-years-old): the AZA group (cases 1–3) had taken in most occasions, 1 mg/kg/day AZA for 8–47 weeks; CYC group (cases 4–11), 1 mg/kg/day CYC for 4–20 weeks; CYC plus AZA group (cases 12–13), the same doses as above for 5–7 weeks; and CYC plus PS-K group (cases 14–20), 3 g orally/day PS-K and 1 mg/kg/day CYC for 4–28 weeks. There were 12 cases of nephrotic syndrome, 5 cases of nephritis, and 1 each of progressive systemic sclerosis, systemic lupus erythematosus, and juvenile rheumatoid arthritis.

Blood samples were obtained from the patients 4–5 hours after taking of the agents. Peripheral lymphocytes from each of the controls and the patients were cultured in the Gibco chromosome medium containing 5-bromo-2'-deoxyuridine (BUdR, 30 μ g/ml), at 37°C for 72 hours in complete darkness. Two hours before harvest, cultures were treated with 0.4 μ g/ml of colcemid followed by hypotonic treatment (0.075 M KCl, 12 min), fixed in 3 : 1 methanol-acetic acid, and air-dried on slide. The slides were mounted with Sørensen buffer (pH 7.0) for 15 min, stained successively with Hoechst 33258 (1.0 μ g/ml in the same buffer) for 12 min, and rinsed briefly in the buffer. They were mounted in the buffer under a coverslip and placed in a petri dish. Photosensitization was done at a distance of 10 cm from the source of 200 W mercury arc lamp for 15 min. The slides were incubated for 15–30 min in 2 $\times$ SSC at 60°C, followed by 30-min staining in 3% Giemsa solution (Giemsa staining buffer, pH 6.8). For each culture 9–40 well-spread metaphases were selected under 1,000 $\times$ magnification and examined for SCEs.

RESULTS

In the control group, a total of 500 cells were analyzed for the frequency of SCEs (Table 1). The mean frequency was 5.63 ± 0.587 SCEs per cell (mean $\pm$ SD), with a range from 3.61 to 7.76. The frequency in the AZA group ranged from 11.27 to 12.24, with a mean value of 11.70. The frequency in CYC group ranged from 10.20 to 18.03, with a mean value of 15.10 ± 2.431 . A highly significant increase was observed in CYC group. The CYC plus AZA group showed an even higher value (19.52). The frequency in CYC plus PS-K group ranged from 6.24 to 12.44, with a mean value 9.16 ± 2.225 .

Figure 1 shows the relation between frequency of SCEs and medication term in these four groups. It was found that the SCE frequency in the CYC plus PS-K group was lower than that in other groups at any time of medication. And it ap-

Table 1. Frequency of SCEs in treated and non-treated groups.

Case No.	Age	Sex	Diagnosis	Therapy	AZA and/or CYC		Total cells counted	Total SCEs	SCEs/cell	Mean±SD
					Dose	Term				
Controls										
1	8	F	JRA	AZA PRE	1,680 mg	8 W	500	2,817		5.63±0.587
2	13	F	PSS	AZA PRE	19,740 mg	47 W	40	451	11.27	*1
3	10	F	NPS	AZA PRE	2,520 mg	9 W	25	306	12.24	
							10	116	11.59	
4	14	F	SLE	CYC PRE	1,400 mg	4 W	9	134	14.88	
5	5	M	NPS	CYC PRE	1,120 mg	4 W	30	541	18.03	
6	9	F	PLN	CYC IND	840 mg	4 W	25	334	13.36	
7	12	F	PUN	CYC IND	1,400 mg	5 W	25	255	10.20	
8	14	F	PLN	CYC IND	9,800 mg	20 W	25	427	17.08	
9	10	M	NPS	CYC IND	4,200 mg	12 W	25	408	16.32	
10	14	F	PLN	CYC IND	3,500 mg	14 W	16	249	15.56	
11	8	M	PLN	CYC IND	1,540 mg	9 W	25	384	15.36	
12	13	M	NPS	CYC PRE AZA	2,100 mg 2,100 mg	5 W 5 W	10	196	19.64	*2
13	13	F	NPS	CYC PRE AZA	1,813 mg 1,813 mg	7 W 7 W	10	194	19.41	
14	6	M	NPS	PS-K CYC PRE	560 mg	4 W	25	156	6.24	
15	14	F	NPS	PS-K CYC PRE	1,680 mg	4 W	25	167	6.68	
16	10	M	NPS	PS-K CYC PRE IND	700 mg	4 W	13	114	8.77	
17	10	F	NPS	PS-K CYC PRE	1,260 mg	6 W	25	275	11.00	
18	6	M	NPS	PS-K CYC PRE	3,920 mg	28 W	25	225	9.00	
19	9	M	NPS	PS-K CYC PRE	3,360 mg	12 W	25	311	12.44	
20	9	M	NPS	PS-K CYC PRE IND	2,772 mg	12 W	24	240	10.00	
										9.16±2.225

JRA, juvenile rheumatoid arthritis; PSS, progressive systemic sclerosis; NPS, nephrotic syndrome; SLE, systemic lupus erythematosus; PLN, prolonged nephritis; PUN, purpura nephritis; AZA, azathioprine; PRE, prednisolone; CYC, cyclophosphamide; IND, indomethacin.

*1. There is a significant difference between CYC group and normal controls ($p < 0.001$).

*2. There is a significant difference between CYC group and CYC plus PS-K group ($p < 0.001$).

Fig. 1. Relation between SCEs and medication term. Ordinate: SCEs per cell. Abscissa: time of medication (week). △, cyclophosphamide; □, azathioprine; ■, cyclophosphamide combined with azathioprine; ▲, cyclophosphamide combined with PS-K.

Fig. 2. SCEs after cessation of cyclophosphamide medication. Ordinate: SCEs per metaphase. Abscissa: time. Four cases of cyclophosphamide group were examined for frequency of SCEs after cessation of medication. Frequency of SCEs returned to almost normal levels 3-4 months later.

peared that the frequency of SCEs was not medication term-dependent in these four groups, when each immunosuppressive agent was prescribed with uniform doses such as 1 mg/kg/day.

As seen in Fig. 2, high frequency of SCEs which was detectable during the treatment of CYC medication returned to almost normal levels at 3-4 months after the cessation of CYC medication.

DISCUSSION

Recently, undesirable side-effects of immunosuppressive agents have been reported by several authors, and attention has been called to higher risk of malignant disease (Wegmann *et al.*, 1971; Manny *et al.*, 1972). Mutagenic effects of CYC, especially on chromosomal aberrations, have been studied in patients with malignant tumors and other diseases (Vormittag, 1974; Dobos *et al.*, 1974). AZA is also known to induce chromosome aberrations (Zeuthen and Friedrich, 1971; Ganner *et al.*, 1973). Furthermore, possible teratogenicity of CYC and AZA have been reported (Chaube and Murphy, 1968; Cahen, 1966; Rosenkrantz *et al.*, 1967; Tallent *et al.*, 1970; Greenberg and Tanaka, 1964).

The clastogenic effects of these immunosuppressive agents have been studied mostly in patients with malignant tumors or those operated upon organ transplantation. However, little work has been done on the effects upon SCEs of non-tumorous children. In this investigation, the frequency of SCEs in children treated with immunosuppressive agents increased in the following sequence: AZA group, CYC group, and CYC plus AZA group. It seems that the extremely high value observed in the last group is due to the summation or potentiation of the effects exerted by the two agents. A similar result was obtained from a nephrotic child treated with CYC and anticonvulsant drugs (Torigoe, 1979). In all cases dealt in the present study, prednisolone (PRE) or indomethacine (IND) was used in combination with immunosuppressive agents. A question may arise as to whether PRE or IND has influence on the frequency of SCEs or not. Since the cessation of only CYC medication caused the reduction of SCEs to normal level, it is supposed that PRE and IND have no effect on the frequency of SCEs.

PS-K was found to possess an antitumor activity against several experimental tumors, restoring the suppressed delayed-type hypersensitivity reaction in tumor-bearing animals to the normal levels, stimulating humoral immunity in experimental animal, and having a mitogenic effect on human lymphocytes *in vitro* (Tsukagoshi and Ohashi, 1974; Usui *et al.*, 1976; Nomoto *et al.*, 1975; Nakao *et al.*, 1973; Ohono *et al.*, 1976). This drug was found to have no teratogenic activity nor significant side effect on offsprings of rodent species (Seto *et al.*, 1975a, b). No abnormality of chromosomes caused by PS-K was found in mouse bone marrow cells *in vivo* and *in vitro* (Kureha Chemical Industry, 1977). In the present study the frequency of SCEs decreased markedly, when PS-K was combined with CYC. A similar result was obtained in bone marrow cells of ddY mice (Torigoe *et al.*, unpublished data). It is known that mutagen increases the frequency of SCEs (Perry and Evans, 1975). It may not be always true that reduction of SCEs means decrease in mutagenicity. Though there are no data available at present to clarify the mechanism is involved in the effect of PS-K, it is possible that PS-K may reduce a mutagenicity afforded by treatment with CYC.

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