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CHROMOSOMAL STUDY OF PATIENTS WITH PSORIASIS TREATED WITH AMETHOPTERIN

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Abstract. A chromosomal study was carried out in twenty randomly selected patients with psoriasis while being treated with large doses of amethopterin for long periods of time (total dose from 75 mg to 6.7 g). A cytogenetic micro-tecnique was applied to peripheral blood cultures. The study failed to reveal any significant increase in the number of chromosomal breaks or aneuploid cells in these patients' leukocytes.

In recent years, chromosomal changes following the use of certain drugs have been observed. The alterations range from single chromatid breaks to isochromatid breaks, deletions, and exchange figures. Although the significance of these findings is still unclear, the possibility of a teratogenic effect of such chemicals as Lysergic Acid Diethylamide (LSD) (1, 2) and antimetabolites (3) has been considered.

Ryan, Boddington & Spriggs (8) and more recently, Mogens Krogh Jensen (5) and H. Locher & J. Franz (6) have reported chromosomal abnormalities in peripheral blood cultures from psoriatic patients treated with aminopterin and/or amethopterin. These anti-folic acid agents are considered the most effective systemic drugs in the management of psoriasis unresponsive to the conventional methods of therapy. Since many of these patients are young, including women of childbearing age (with added risk of teratogenic effect on their offspring), it seemed important to undertake a cytogenetic study of a group of patients treated with anti-metabolites.

MATERIALS AND METHODS

Fourteen female and six male randomly selected patients with psoriasis were studied (Table I). Their ages ranged from eighteen to sixty-nine years. Amethopterin (Methotrexate, Lederle) was administered orally to four and parenterally to sixteen patients. The following dosage schedules were employed: 1) Twenty-five mg amethopterin intramuscularly once weekly. The solution was prepared by adding four ml distilled water to a vial of 50 mg amethopterin. Two ml of this solution was injected. 2) Fifty mg amethopterin intramuscularly every other week. 3) Twenty-five mg (ten tablets) amethopterin orally in one single dose, once weekly. 4) Five mg amethopterin orally, daily for four days, followed by three free days. Duration of treatment varied from four to two-hundred forty-five weeks. In seven patients (nos. 1, 5, 8, 12, 13, 15 and 20 in Table I), periods of remission, varying from two to four months occurred. During remission, amethopterin was not administered. The total dose administered varied from a minimum of seventy-five mg, to a maximum of 6.7 g. The time interval between the last dose of amethopterin received by the patient and the start of the chromosomal study varied from one to twenty-four days. During the administration of amethopterin, none of the patients received any ionizing radiation. Three patients, however, had received a total of 1000 R grenzray radiation four years prior to the administration of amethopterin. One patient was treated with arsenicals some twenty years ago.

The cytogenetic micro-tecnique as described by Valenti & Vetharany (11), was applied to periopheral blood cultures. No failure in culture of leukocytes was encountered in the present study. Chromosome preparations on slides were scanned under oil immersion ($\times$ 1000).

A total of 940 cells from the twenty patients were analyzed, averaging forty-seven cells per patient. For each patient, thirty-six cells were photographed, the negative films studied on a robot projector (11), and three to five cells were karyotyped (see representative karyotype—Fig. 1).

RESULTS

The incidence of chromosomal breaks was less than two percent, equaling the usual incidence ob-

Table I. *Results of cytogenetic study of peripheral blood cultures from psoriatic patients treaeted with amethopterin*

Patients			Methotrexate treatment					Cells analyzed		Chromosomal count				
No.	Age	Sex	Oral	Parenteral	Period (weeks)	Total dose (mg)	Last dose[a] (days)	No.	Kar.[b]	>45	45	46	47	<47
1	64	F		Parenteral	185	5200	1	50	4		2	48		
2	43	M		Parenteral	8	150	3	50	5			50		
3	39	F		Parenteral	45	1200	17	45	4		1	44		
4	60	M		Parenteral	10	255	8	45	3		2	43		
5	37	M		Parenteral	245	6700	7	50	3		2	47	1	
6	58	F		Parenteral	4	100	4	50	5			49	1	
7	31	F		Parenteral	4	75	24	50	4		1	49		
8	46	F		Parenteral	140	3800	4	50	3		2	48		
9	55	F		Parenteral	26	625	3	50	3		2	47	1	
10	40	F		Parenteral	16	350	4	50	5			50		
11	73	F	Oral		24	480	3	40	3	1		38	1	
12	41	F		Parenteral	142	3600	4	50	3			49	1	
13	18	F	Oral		165	3800	4	50	3		2	78		
14	22	M	Oral		14	375	3	50	3		1	49		
15	54	F		Parenteral	160	4600	3	40	3		1	39		
16	62	M		Parenteral	10	200	4	40	3			40		
17	49	F		Parenteral	9	225	5	40	3			39	1	
18	37	F		Parenteral	34	900	5	40	3			39	1	
19	36	M		Parenteral	24	625	3	50	3			50		
20	69	F	Oral		70	1800	5	50	3	1		48	1	

[a] Time interval between last dose and chromosomal study.
[b] Kar. = Karyotype.

served in this laboratory in normal subjects. Most of the observed breaks, actually still showed some alignment of the fragments and, therefore, could be considered gaps and not true breaks. In twenty-six cells, aneuploid numbers of chromosomes were noted. The incidence was six percent in two patients, four percent in six patients, and two percent in seven patients. Different chromosomes were involved in each anomalous karyotype. All the aberrations noted are to be considered artifactual. Findings are summarized in Table I.

DISCUSSION

Chromosomal damage due to antimetabolites such as 5-fluorodeoxyuridine, triethylenemelamine, and triethylenthiophosphoamid has been demonstrated by in vitro experiments by Hampel, Kober, Rösch, Gerhartz & Meinig (3). Breakage at specific chromosome sites due to bromodeoxyuridine was noted by Hsu & Sommers (4). Attempts by Hampel and co-workers (3), however, to induce similar in vitro changes with extremely high doses of amethopterin (0.1–100 μg/ml culture medium) have failed, as have in vivo experiments in rats.

Taylor (10) has described chromatid breaks induced by aminopterin in growing roots of vicia faba. Ryan, Boddington & Spriggs (8) studied sixteen patients with psoriasis, eight of whom were treated with antimetabolites. Of this latter group, while three patients received aminopterin only, five patients received both aminopterin and amethopterin; it is possible that the chromosomal changes observed in these five patients were induced by aminopterin rather than amethopterin. As to the three patients who did not receive aminopterin, one may conjecture that either some factors acting in the in vitro environment may have been responsible for the chromosomal damage, or that the patients' cells were particularly sensitive to the effect of amethopterin. Similar interpretations have been offered to explain the discrepanicies between positive (1, 2) and negative findings (7, 9) in cytogenetic studies carried out in LSD "users".

Furthermore, all patients studied by Ryan and co-workers (8), were exposed to diagnostic radiation when X-ray screening was performed for positioning of a Crosby capsule in the jejunum, and radiation has been demonstrated to damage chro-

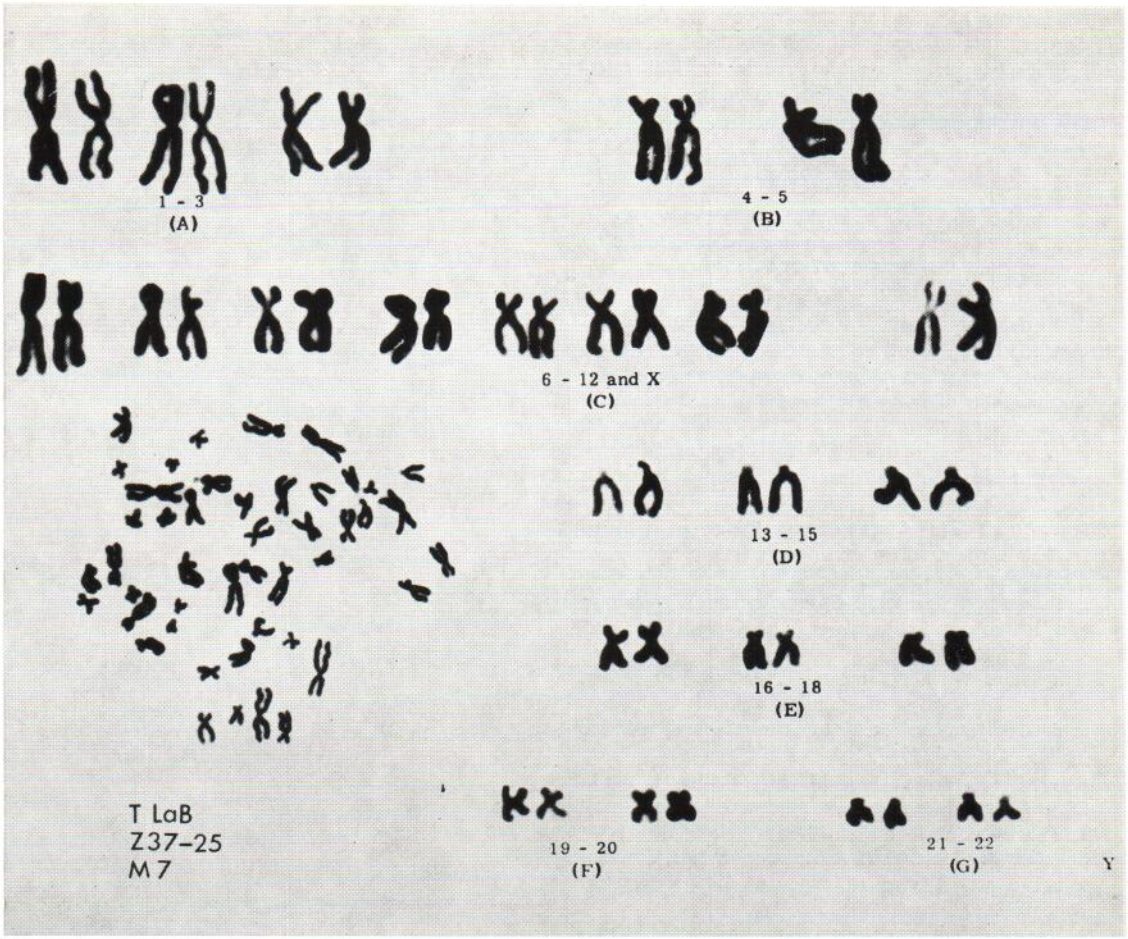

Fig. 1. Representative 46: × ×, Karyotype of case no. 7, Table I.

mosomes of lymphocytes, both in vitro and in vivo, even at the low diagnostic dosage range.

More recently, Mogens Krogh Jensen (5) studied ten patients. Six were treated with azathioprine (Imuran, Burroughs–Wellcome) and only four patients, all afflicted with psoriasis, received amethopterin. The total dosage of amethopterin given prior to the cytogenetic studies was considerably lower than that used by Ryan (8) or by the authors of the present study. Mogens Krogh Jensen (5) confirmed Ryan's findings concerning structural chromosomal aberrations. However, he was unable to demonstrate an increased number of aneuploid cells. H. Locher & J. Franz (6) investigated twelve patients; all were treated with amethopterin and the total dosage used by these authors generally exceeded that used by Mogens Krogh Jensen (5). In this study, four patients showed increased incidence of chromosomal breaks, and two exibited aneuploid cells.

Patients studied in this series were treated with amethopterin for long periods of time, and with relatively large doses reaching a total of 6.7 g in one patient. Yet, the frequency of chromosomal breaks and gaps observed was insignificant. In twenty-six cells, of a total of nine-hundred forty cells analyzed, aneuploid numbers of chromosomes were noted; however, because of their low incidence (less than three percent), the significance of these findings is questionable.

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REFERENCES

1. Cohen, M. M., Hirschhorn, K. & Frosch, W. A.: In vivo and in vitro chromosomal damage induced by LSD-25. New Eng J Med *277:* 1044–1049 (Nov.) 1967.
2. Egozcue, J., Irwin, S. & Maruffo, C. A.: Chromosomal damage in LSD users. JAMA *204:* 214 (April), 1968.
3. Hampel, K. E., Kober, B., Rösch, D., Gerhartz, H. & Meinig, K. H.: The action of cytostatic agents on the chromosomes of human leukocytes in vitro (preliminary communication). Blood *27:* 816, 1966.
4. Hsu, T. C. & Sommers, C. E.: Effect of 5-bromodeoxyuridine on mammalian chromosomes. Proc Nat Acad Science. Wash *47:* 396, 1961.
5. Jensen, M. D.: Chromosome studies in patients treated with azathioprine and amethopterin. Acta Med Scand *182:* 4, 1967.
6. Locher, H. & Franz, J.: Chromosomenveränderungen bei Methotrexatbehandlungen. Med Welt *31:* 1965 (August), 1967.
7. Loughman, W. D., Sargent, T. W. & Israelstam, D. M.: Leukocytes of humans exposed to lysergic acid diethylamide: lack of chromosomal damage Science *158:* 508, 1967.
8. Ryan, T. J., Boddington, M. M. & Spriggs, A. I.: Chromosomal abnormalities produced by folic acid antagonists. Brit J of Derm *77:* 555 (Nov.), 1965.
9. Sparkes, R., Melnyk, J. & Bozzetti, L. P.: Chromosomal effect in vivo of exposure to lysergic acid diethylamide. Science *160:* 1343, 1968.
10. Taylor, J. Herbert: Effects of inhibitors of thymidylate synthetase on chromosome breakage and reunion. Exp Cell Res, Suppl. *9:* 99, 1963.
11. Valenti, C. & Vethamany, K. V.: Functional anatomy of a cytogenetic service. Amer J Obstet Gynec *99:* 434 (Oct.), 1967.

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