Chromosome damage after intra-articular injections of radioactive yttrium

Effect of immobilization on the biological dose

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Favourable results have been obtained in the treatment of persistent joint effusion with radioactive agents (*e.g.* Ansell, Crook, Mallard, and Bywaters, 1963; Virkkunen, Krusius, and Heiskanen, 1967; Delbarre, Cayla, Roucayrol, Menkes, Aignan, and Ingrand, 1970; Oka, Rekonen, Ruotsi, and Seppälä, 1971). The future of this kind of radiotherapy is largely dependent on whether the ionizing radiation has side-effects. Chromosome damage in patients who had received intra-articular injections of radioactive gold (^{198}Au) has recently been reported (Stevenson, Bedford, Hill, and Hill, 1971a).

At present radioactive yttrium (^{90}Y) is displacing ^{198}Au as an agent for radiation synovectomy. In radiophysical characteristics ^{90}Y is clearly superior to ^{198}Au . It emits only beta-rays with a high maximum energy (2.25 MeV) and a maximum range in the tissue of 11 mm. (average 4 mm.). The corresponding figures for ^{198}Au indicate that it is much less effective. The two isotopes have the same half-life, 2.7 days.

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We have used intra-articular injections of ^{90}Y resin colloid for the treatment of rheumatoid knee joints. In our studies an appreciable accumulation of ^{90}Y could be shown in the regional lymph nodes in some cases, but when rest in bed was instituted after injection, the leakage of radioactivity was significantly reduced. The average dose absorbed in the lymph nodes diminished from 1600 rad to 200 rad (Oka and others, 1971; Rekonen, Oka, and Ruotsi, 1972).

In the present investigation chromosome damage in circulating lymphocytes from the peripheral blood was studied after intra-articular yttrium injections in two groups of patients with rheumatoid arthritis, unimmobilized and immobilized.

Patients and methods

The series comprised 25 rheumatoid arthritis patients who had received intra-articular injections of radioactive yttrium into the knee joint. ^{90}Y resin colloid (Radiochemical Centre, Amersham, England; colloid size 400 Å)

Table I A Data on eleven patients who were not immobilized after ^{90}Y treatment

Case No.	Age (yrs)	Sex	Duration of disease (yrs)	Dose (mCi)	Time to test (mths)	No. of cells analysed	No. of cells with aberrations				Radiation dose in lymph nodes (rad)	Phenylbutazone
							Dicentric + rings	Fragments	Cs	Total (Cu + Cs)		
1	32	F	2	6	12	103	0	0	0	0	3,600	+
2	42	M	1	12	12	40	1	0	0	1	1,600	+
3	60	F	1	6	12	100	0	1	0	1	3,200	+
4	53	F	32	6	10	100	0	3	0	3	1,000	+
5	57	F	6	5	17	100	1	5	0	6	1,200	+
6	38	F	2	5	16	100	0	2	0	2	280	+
7	48	M	2	4	15	100	2	3	0	5	440	+
8	51	F	18	5	17	100	1	1	0	2	320	+
9	69	F	3	5	17	100	1	1	1	3	1,600	+
10	77	F	3	10	10	28	0	1	1	2		+
11	64	F	10	6	10	100	0	4	0	4	600	+
Mean	54		8.8	6.4	13.5	88	0.5	1.9	0.2	2.6	1,400	

was injected together with hydrocortisone acetate and lidocaine. The dose injected was 4–6 mCi, except in two cases in which two separate yttrium injections were given. After injection, accumulation of ^{90}Y in the inguinal lymph nodes was measured and the corresponding radiation doses calculated.

The series was divided into two groups: The patients of the first group were not immobilized after treatment (Table IA). The patients of the second group were kept in bed for three days after isotope injection (Table IB). The control group consisted of fourteen unselected, consecutive patients suffering from rheumatoid arthritis (Table IC). Patients who had been given radiation therapy or immunosuppressive drugs were excluded from the study. Chromosomal aberrations after azathioprine administration have been demonstrated by Krogh Jensen (1967). It has also been shown that chromosome damage occurs in patients taking phenylbutazone (Stevenson, Bedford, Hill, and Hill, 1971b). For years phenylbutazone has been in extensive use as an antirheumatic agent in Finland. Nearly all the treated patients as well as our controls had taken this drug for shorter or longer periods,

but it proved impossible to collect accurate data on the total dose or length of administration. However, the virtual absence of chromosomal aberrations in the control group suggests that the effect of phenylbutazone on our results is not of crucial importance.

For a detailed account of the types of chromosome aberrations and the rationale of interpretation, readers are referred to the extensive description by Stevenson and others (1971b). Peripheral blood was cultured in the usual way (Moorhead, Nowell, Mellman, Battips, and Hungerford, 1960). The culture time was 48 hrs with colchicine added 3 hrs before harvest. The cells were placed in hypotonic solution for 10 min. in 0.075 N KCl and fixed in 3:1 methanol-acetic acid. The cells were air-dried on slides and stained with Giemsa.

The aim was to analyse 100 mitoses from each patient, but this was not always possible. Only cells of good technical quality and having 46 or 45 centromeres were chosen for analysis. The following aberrations were scored separately: dicentric, rings, fragments, and structural rearrangements. No multicentric were encountered. Since individual chromosomes were not

Table IB Data on fourteen patients who were immobilized after ^{90}Y treatment

Case No.	Age (yrs)	Sex	Duration of disease (yrs)	Dose (mCi)	Time to test (mths)	No. of cells analysed	No. of cells with aberrations				Radiation dose in lymph nodes (rad)	Phenylbutazone
							Dicentric + rings	Fragments	Cs	Total (Cu + Cs)		
1	37	F	2	6	10	95	3	1	1	5	200	+
2	42	F	9	6	11	32	0	0	0	0	320	?
3	57	F	12	6	8	100	0	0	0	0	200	+
4	24	F	1	6	11	101	0	0	0	0	320	+
5	26	F	1	6	7	67	0	0	0	0	0	+
6	32	F	6	6	4	31	0	1	0	1	80	—
7	57	F	5	6	3	100	1	1	1	3	280	+
8	38	F	11	6	4	111	0	1	0	1	200	+
9	28	F	11	6	6	65	0	0	0	0	240	—
10	60	F	2	6	3	31	0	1	0	1	240	+
11	33	F	10	6	5	72	1	3	0	4	920	+
12	58	F	2	6	8	67	1	0	0	1	480	+
13	58	F	7	6	5	48	0	0	0	0	200	+
14	37	M	1	6	2	100	0	1	0	1	120	+
Mean	40		5.7	6	6.2	73	0.4	0.6	0.1	1.2	270	

Table IC Data on thirteen control patients who were not treated with radioisotopes

Case No.	Age (yrs)	Sex	Duration of disease (yrs)	No. of cells analysed	No. of cells with aberrations				Phenylbutazone
					Dicentric + rings	Fragments	Cs	Total (Cu + Cs)	
1	54	F	5	100	0	0	0	0	+
2	45	F	6	100	0	1	0	1	+
3	38	F	1	100	0	0	0	0	—
4	49	F	3	43	0	0	0	0	+
5	63	M	18	100	0	1	0	1	+
6	50	F	10	100	0	0	0	0	+
7	61	F	1	43	0	1	0	1	+
8	25	F	1	34	0	0	0	0	+
9	62	F	2	100	0	1	0	1	+
10	56	F	1	100	0	0	0	0	+
11	49	F	3	100	0	0	0	0	+
12	59	F	7	53	0	0	0	0	+
13	54	F	26	100	0	1	0	1	+
Mean	52		6.3	83	0	0.4	0	0.4	

measured, only structural rearrangements in which the length of a given chromosome arm was increased or decreased by about 30 per cent. or more were scored. With these criteria many translocations obviously go undetected. However, in view of the well-known observation that homologous chromosomes appear to be of different length even within cells, it was decided to adopt these stringent criteria rather than to take the risk of including uncertain translocations.

In the text and Tables, dicentrics and rings have been considered together and fragments separately. All these constitute unstable aberrations (Cu). Cs denotes stable structural aberrations, *e.g.* translocations, duplications, and deletions.

In addition, gaps and chromatid breaks were scored. Since they were few in number and equally distributed in the three groups of patients, they will not be dealt with further.

Results

The patients in the immobilized group were somewhat younger (average 40 yrs) than those in the other groups (averages 54 and 52 yrs, respectively). No differences in the prevalence of Cu or Cs aberrations have been noted in different age groups (UNSCEAR, 1969). The time interval between ⁹⁰Y treatment and chromosome investigation differed markedly between the nonimmobilized and immobilized cases, being about twice as long (13 months) for the former as for the latter (6 months). Since the decrease in the numbers of Cu cells with time after irradiation is roughly exponential (Buckton, Jacobs, Court Brown, and Doll, 1962), more aberrations had obviously been eliminated in the patients who were not immobilized. Hence the higher prevalence of aberrations in these patients (see below) is an underestimate, and the real differences were still greater. The number of cells analysed in the two groups did not differ significantly.

The dose administered was closely similar in the two groups (means 6.4 mCi in the nonimmobilized and 6.2 mCi in the immobilized patients). However, the radiation doses in the inguinal lymph nodes ranged from 280 to 3,600 rad (average 1,400) in the nonimmobilized group, and from 0 to 920 rad (average 270) in the immobilized. The difference is highly significant.

Table II Breakdown of cytogenetical findings into prevalence of aberrations and prevalence of cells with any aberration

Treatment group	No. of cases	No. of cells studied	Prevalence of aberrations				Prevalence of cells	
			Dicentrics + rings		Fragments		Cu + Cs	
			No.	per 100 cells	No.	per 100 cells	No.	per 100 cells
⁹⁰ Y treated								
Not immobilized	11	971	7	0.72	29	2.99	29	2.99
Immobilized	14	1020	7	0.69	11	1.08	17	1.67
Controls	13	1073	0	0	7	0.65	5	0.47

The prevalence of significant chromosomal aberrations (dicentrics, rings, and fragments) was increased over the controls in both groups of treated patients (Tables I and II). This is seen to be true both from the number of aberrations per cell and from the proportion of cells with some abnormality. The abnormalities were particularly frequent in the non-immobilized patients, only one of whom had no abnormal cells, while six of the immobilized patients and eight of the controls had no abnormal cells (Table III).

Table III No. of patients with and without any cytogenetically abnormal cell

Treatment group	Chromosome damage: No. of cases		
	With Cu + Cs	Without Cu + Cs	Total
⁹⁰ Y treated			
Not immobilized	10	1	11
Immobilized	8	6	14
Controls	5	8	13

Statistical studies revealed a significant difference between the nonimmobilized group and the controls, but not between the latter and the immobilized group (Table IV).

Table IV Statistical comparison of the patient groups (*P* values)

Groups compared	Cells with dicentrics + rings	Cells with fragments	Cells with Cu + Cs
Not immobilized vs. Immobilized	—	0.05	—
Not immobilized vs. Controls	0.05	0.01	0.001
Immobilized vs. Controls	—	—	—

No correlation could be established between the individual radiation doses to the inguinal lymph nodes and the prevalence of chromosomal aberrations.

Discussion

The number of stable chromosome rearrangements (Cs) consisting of translocations, duplications, deletions, etc., was very low in our series compared with those of others. This may be because we adopted stringent criteria for the identification of such rearrangements. As a consequence, rearrangements of stable type (Cs) and unstable type (Cu) were considered together in the final evaluation of the results.

The three groups of patients were compared with regard to both the incidence of cells with any abnormality and the incidence of abnormalities. In the control group aberrations were clearly less frequent than the other two groups. The only type of aberration seen in the control group was fragments; dicentrics and rings were not encountered at all. Dicentrics are, in fact, very rare in unirradiated subjects (UNSCEAR, 1969). There were some dicentrics plus rings in the treated groups (0.69 and 0.72 per cent.). Statistically, the control group differed significantly from the nonimmobilized group but not from the immobilized group. However, although the immobilized group had fewer cells with aberrations (1.67 per cent.) than the nonimmobilized group (2.99 per cent.), the difference was not statistically significant. The difference in the frequency of fragments (1.08 versus 2.99 per cent.) was almost significant. In this context it should be borne in mind that the time interval between the radioactive treatment and the test was twice as long in the case of the nonimmobilized patients, which biases the findings (*cf.* Results).

Our results clearly indicated that chromosome damage can be produced by intra-articular injection of radioactive yttrium. However, there appears to be much less chromosome breakage after this treatment than after treatment with radioactive gold. The frequency of damaged cells in patients given intra-articular injections of ^{198}Au was 8.5 per cent., including dicentrics in 7.1 per cent. (Stevenson and others, 1971). The corresponding figures in our ^{90}Y -treated patients were considerably lower. Yet the cytogenetical methods and criteria of interpretation were almost identical.

The dose of radioactivity was about three times higher with gold than with yttrium while the time interval between the treatment and the leucocyte culture was far longer in the gold study. The favourable radiation properties of yttrium allow lower optimal therapeutic doses to be used (Oka and others, 1971). The present study showed that there was correspondingly less biological damage.

The marked chromosome-damaging effect of radioactive gold is not surprising in view of the high doses of radiation found beyond the injected joint in many cases (Virkkunen, Krusius, and Heiskanen,

1967). ^{90}Y is much less dangerous in this respect. In addition, we have previously shown that the spread of radioactivity outside the injected joint can be successfully reduced by immobilizing the joint. In this paper, the favourable biological effect of this procedure is demonstrated. Our patients were kept in bed for 3 days after isotope injection. At present we are applying splints to prevent any movement of the joint.

Most of the patients treated with intra-articular yttrium had long-standing active synovitis with large effusions. In synovial biopsies taken for electron microscopy a few days after isotope injection, the ^{90}Y particles were found in vacuolar cavities among the intercellular ground substance. This was considered to be the route by which ^{90}Y leaks into the lymph stream (Isomäki, Inoue, and Oka, 1972).

As the risk of chromosomal damage had no clear relationship to accumulated radiation dose in inguinal lymph nodes, it seems reasonable to assume that damage to lymphocytes occurred not only in the regional lymph nodes but also in circulating cells passing through the knee region. A distinctive feature of active rheumatoid synovitis is active circulation. This may increase the proportion of lymphocytes exposed at the site of injection. Inter-individual variation in the size of the popliteal lymph nodes and their drainage are also possible. Furthermore it should be remembered that the number of antigenic stimuli received by the patient between the time of exposure and the time of sampling may affect the rate of elimination of damaged cells (Nowell, 1965).

Intra-articular yttrium resin colloid with large particle size is at present the method of choice for radiation synovectomy in large joints. Young patients should not be treated with radioactive agents without strong clinical indications. In such cases chemical synovectomy with osmic acid or surgical synovectomy should always be tried first. Moreover, the isotope doses can be reduced below the conventional level.

Summary

Chromosome studies were performed on peripheral blood lymphocytes from 25 patients with rheumatoid arthritis after intra-articular injections of radioactive yttrium. ^{90}Y injections were shown to produce chromosome breakage. There were fewer aberrations in the patients who were immobilized for 3 days after ^{90}Y treatment. The percentage of chromosome rearrangements after ^{90}Y treatment was 2.99 (dicentrics 0.72) in the eleven nonimmobilized patients and 1.67 (dicentrics 0.67) in the fourteen who were immobilized. The corresponding figure in a control group consisting of thirteen patients with rheumatoid

arthritis who had not received ^{90}Y was 0.47 (dicentric/0).

We conclude that immobilization is an effective means of reducing biological radiation damage in this type of treatment. When our figures are compared with the corresponding figures for ^{198}Au -treated patients reported by others, the prevalence of chromosome aberrations appears to be much lower

with ^{90}Y . This is because far smaller optimum doses are required.

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