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Persistent chromosomal aberrations in somatic cells in testicular cancer patients after different therapies

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Background. *The damage due to radiation or chemotherapeutic agents has been estimated successfully for the last 35 years from the numbers of the chromosome changes. This finding may serve as biological dosimeter. The aim of the study was to find persistent chromosomal aberrations in somatic cells in testicular cancer patients after different therapies.*

Patients and methods. *This prospective study includes 60 patients with testicular tumours. With respect to the histological results and various therapies that they were given they were divided into four groups. Prior to treatment, we did not detect any deviations either in the genome picture of our patients or in that of the subjects of the control group without malignant disease. The changes in the genome of individual cells after therapy were detected by the following tests: structural chromosomal aberrations (SCA) test, sister chromatid exchange (SCE) test and micronucleus (MN) test performed on binuclear lymphocytes.*

Results. *Immediately after the completion of treatment, chromosomal aberrations were inhibited, with the exception of dicentrics which persisted. Chemotherapy is less detrimental to the genome picture than radiotherapy and causes different types of chromosome changes. From the cytologic and mutagenetic points of view, irradiation proved to be more aggressive to patients than chemotherapy. Six months after the completed treatment, the mitotic activity was found to be nearly normal; but the chromosome damage persisted and was higher than before therapy and in the fourth group of patients who had been only operated on.*

Conclusions. *After irradiation as well as after chemotherapy the genome was repaired, because the damaged cells had died away. Considering that in the observed patients, only small tissue-cellular complex responded to radiation and that the number structural chromosome changes, predominantly unstable aberrations, such as dicentrics, was rather high, we assume that the repair of the genome will be faster in these patients.*

Key words: testicular neoplasms – therapy; chromosomal aberations; micronuclei

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Introduction

Patients with seminoma tumours received postoperative irradiation[1,2] or postoperative adjuvant chemotherapy with paraplatinum.[3,4] Patients with nonseminoma tumours may be treated by postoperative chemotherapy with BEPV5 or by surgery alone. The choice of treatment modality depends on the stage of disease, and toxic effects of treatment.[1,6-9]

The damage due to radiation or chemotherapeutic agents has been estimated successfully for the last 35 years from the numbers of the chromosome changes in the culture of lymphocytes in the peripheral blood.[10] The exposure of cells to ionising radiation can be recognised from a higher number of structural chromosome changes. This finding may serve as biological dosimeter. The technique most often used for the identification of these changes uses the following steps: preparing the cells exposed to radiation under in vitro circumstances and counting the chromosomal changes.[11-14]

Patients and methods

Subjects

This prospective study included 60 patients with testicular tumours, aged between 15 and 35 years, who had not been previously treated for any malignant disease. With respect to the histological test results and various therapies applied (surgery, radiotherapy, chemotherapy) we divided them into four groups. Mutagenetic tests were performed at diagnosis, immediately after the completion of treatment and six months after it.

The stage of the disease in patients with testicular tumour was defined according to TNM classification from the year 1992 (29).

Treatment approaches

Group I

All patients in the first group received postoperative irradiation. Radiotherapy was performed by an 8 MeV linear accelerator, using X rays. The irradiated area involved the iliac and paraaortal lymph nodes on the same side as the malignant process. The radiation field comprised an area from Th 10 to L5 and had a width of 10-11cm, AP and PA, and the inguinal lymph nodes on the same side, from the upper edge of L5, 1 cm medially, involving also the entire postoperative scar only up to a depth of 3 cm and only AP. On the back, the radiation field was the same, extending up to L5 and from there to the sacrococcygeal bone. The total radiation dose for Stage I was 30 Gy and for Stage II 36 Gy; the daily dose was 1.5 Gy. Thirteen patients were irradiated with 30 Gy and only two with 36 Gy.

Group II

The patients of the second group had a postoperative adjuvant chemotherapy with paraplatinum. A single dose (paraplatinum 300-400 mg/m^2) was administered in short infusion. The patients in the second group received one, two or three cycles of paraplatinum in doses from 450 mg to 750mg, depending on the body surface area. The total dose of paraplatinum thus ranged between 450 mg and 2250 mg per individuum.

Group III

Patients with nonseminoma tumours underwent surgery, orchidectomy of the affected testis followed by the adjuvant chemotherapy according to BEPV schedule: cisplatin 20 mg/m^2 i.v., days 1-5; bleomycin 15 mg/m^2 i.v., 1st and 2nd day; etoposide 100 mg/m^2 i.v., days 1-3; - vinblastine 3 mg/m^2 i.v,. 1st and 2nd day.

Patients had two, three or four cycles of chemotherapy according to BEPV schedule. Seven patients had four cycles, four patients had three cycles and four patients had two cycles of chemotherapy.

Group IV
All patients in this group underwent only surgery, i.e. orchidectomy of the affected testis, followed by lymphadenectomy or observation only.

Cytogenetic studies

We used peripheral blood lymphocytes as target material. Blood samples were taken simultaneously for the following three tests.

Structural chromosomal aberrations (SCA)
We used standard in vitro lymphocyte cultures for structural chromosomal aberration analysis. We added 0.3 ml of heparinised whole blood to 5 ml of Chromosome med 1A-GIBCO culture medium. The first in vitro cell division cycle was established with the addition of 5 μg/ml of BrdU-Sigma. The hypotonic procedure was performed with 0.075 mol/l potassium chloride, whereas fixing was carried out in a mixture of glacial acetic acid and methyl alcohol at a ratio of 1:3. The cell suspension was pipetted onto cold glass slides. The specimens were air-dried and stained with Giemsa-Sigma. The maximum analysed for every test subject were the very first 200 in vitro metaphases. The chromosome analysis was carried out exclusively in the metaphases of the first division cycle, identified by homogeneously stained chromosomes. Structural damages to chromosomes were categorised as chromosomal breaks, acentric fragments, dicentrics and ring chromosomes. Gaps were not included in the total number of chromosomal aberrations.[16]

Sister chromatid exchange (SCE)
The same culture medium was used as in the first test. We prepared in dark 72-hour lymphocyte cultures with the addition of 10 μg/ml BrdU and carried out the procedure according to Kato.[17] We analysed 50 cells per subject, counted SCE and presented them as average numbers per cell. The range of SCE frequencies was also recorded for every subject.

Micronucleus test (MN)
For this test, 3 μg/ml of cytochalasin B-Sigma was added to each in vitro lymphocyte culture in the 43rd hour of cultivation. We used the Fenech-Morley method. Hypotonic procedure was omitted. The specimens were stained with May-Grunwald and Giemsa. We analysed the cells with clearly blocked cytokineses (CB cells), i.e. binuclear cells. We examined 500 cells per person and presented the results as the number of micronuclei per 500 CB cells. For technical reasons, the MN test was not performed on the control group – environmental exposure.[18]

Statistical data processing

The median value of all parameters in the four groups of patients and for all three measurements was presented graphically (Windows Microsoft Excel). The changes in the genome picture were analysed for each treatment modality using the overall analysis of all three measurements (Friedman's test)[19] and the analysis of two measurements (Wilcoxon's test).[19] The Kruskal-Wallis (KW) test and Mann-Whitney (MW) test were used for the comparison of the median values of the areas between the two groups; the difference between groups is statistically significant if $p < 0.025$, since Bonferroni's correction must be taken into account.[20] A personal computer and the SPSS programme package (SPSS for Windows, Version 8.0.1) was used for statistical data processing that was carried out at the Institute for Biomedical Informatics of the Faculty of Medicine, University of Ljubljana.

Results

Prior to treatment, we did not detect any deviations neither in the genome picture of our patients nor of the subjects of the control group without malignant disease. (Table 1, Fig. 1-6).

Immediately after the completion of treatment, the mitotic activity was observed as well as a significant increase in the frequency of chromosomal aberrations. In the patients treated with radiotherapy, this was mainly due to an increased number of dicentrics (Fig. 2). Chemotherapy affects the genome to a lesser degree than radiotherapy. Usually, the percentage of structural chromosome changes is higher after chemotherapy ($p = 0.005$) (Fig. 1A), but still lower than after irradiation $p = 0.005$ (Fig. 1). The types of chromosomal changes in the observed patients were different, with the unstable chromosome changes predominating ($p = 0.005$) (Fig. 2). After chemotherapy, the number of dicentrics is not significantly different ($p = 0.08$) (Figure 2). The number of MN is higher after irradiation than chemotherapy ($p = 0.005$) (Fig. 5), although after chemotherapy according to BEPV schedule and chemotherapy with paraplatinum, the numbers of micronuclei differ significantly ($p = 0.005$) (Fig. 5). A significantly higher number of SCE was noted after chemotherapy than after radiotherapy ($p = 0.001$) (Fig. 6).

Six months after the completion of treatment, the mitotic activity was found to be mainly normal, but in comparison to the fourth group that was treated only by surgery, a large percentage of chromosomal aberrations persisted (Fig.1).

The analysis of genome changes immediately after the completion of therapy and six months later showed statistically significant difference in chromosome changes in the first group ($p = 0.01$) and in the third group

Table 1. The median value of all parameters in four groups of patients and for all three measurements

	measurement	group I	group II	group III	group IV
Me of % SCA/200 cells	1	2	1,5	1,5	1,5
	2	50	4,5	6	1,5
	3	9	3	3,5	1,5
Me of dicentrics/200 cells	1	0	0	0	0
	2	17	0	0	0
	3	6	0	0	0
Me of acentric fragments/	1	1	0	0	0
200 cells	2	21	2,5	2	0
	3	8	2	2,5	0
Me of chromosomal breaks/	1	3	3,1	2	2,9
200 cells	2	5	5,1	4,9	3
	3	5	4,1	4	3
Me ofMN/500CB cells	1	5	6	6	4
	2	19	8	12	4
	3	12	7	10	4
SCE/50 cells	1	6,2	6,4	6,4	6,2
	2	6,4	7,9	8,3	6
	3	6,5	7,4	7,2	6

Me = median value, No = number, SCA = structural chromosomal aberrations, MN = Micronucleus test, SCE = sister chromatid exchange

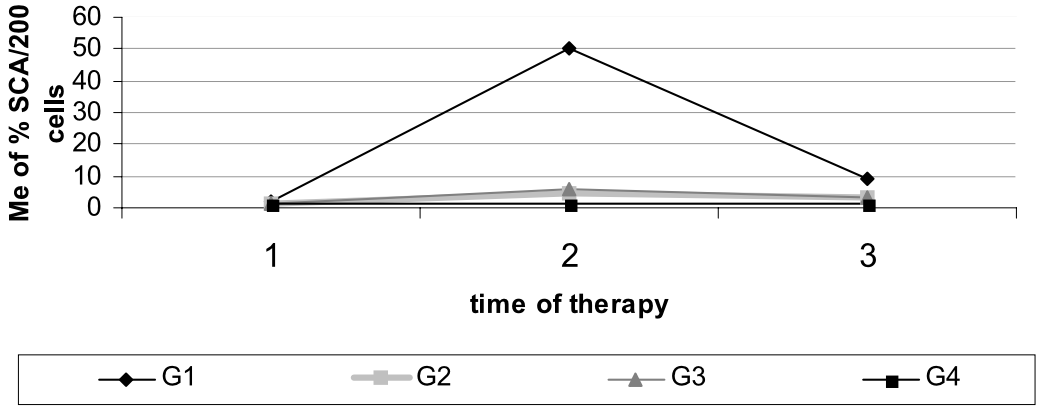

Figure 1. Median value of the chromosomal aberrations of patients with testicular tumour (p = 0.005).

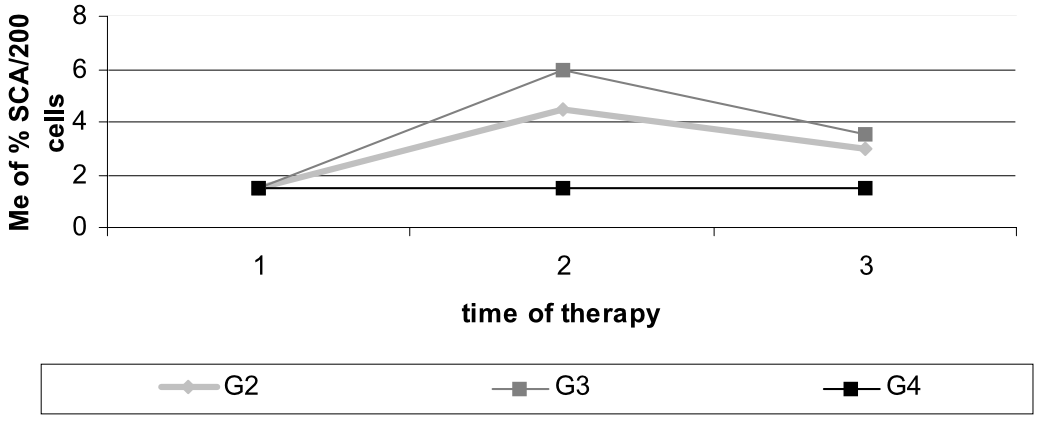

Figure 1a. Median value of the chromosomal aberrations of patients with testicular tumour (p = 0.005).

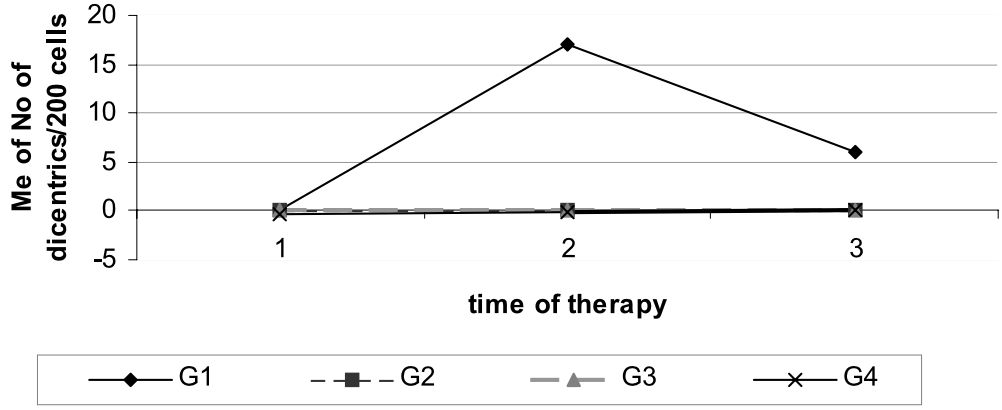

Figure 2. Median value of the dicentrics of patients with testicular tumour (p = 0.005).

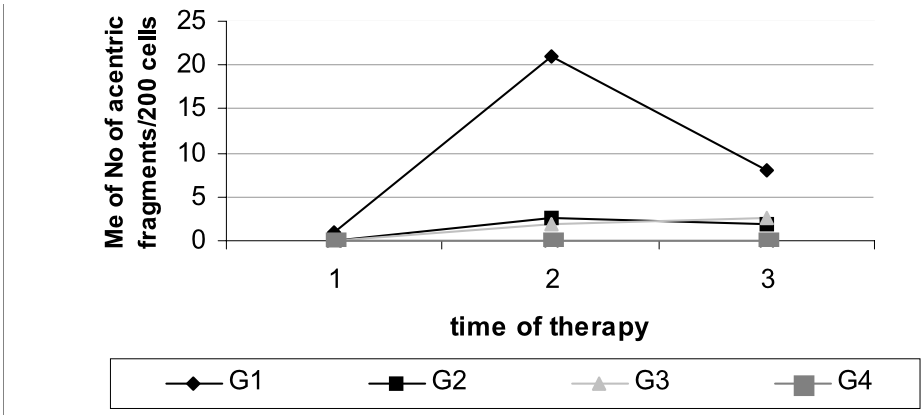

Figure 3. Median value of the acentric fragments of patients with testicular tumour (p = 0.005).

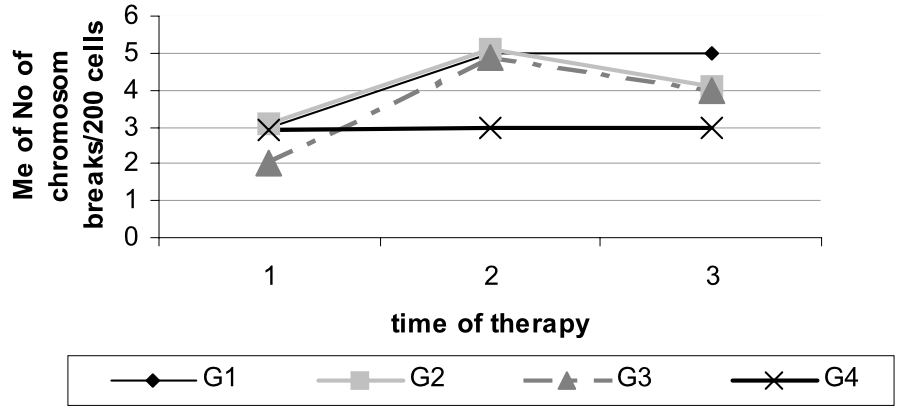

Figure 4. Median value of the chromosomal breaks of patients with testicular tumour.

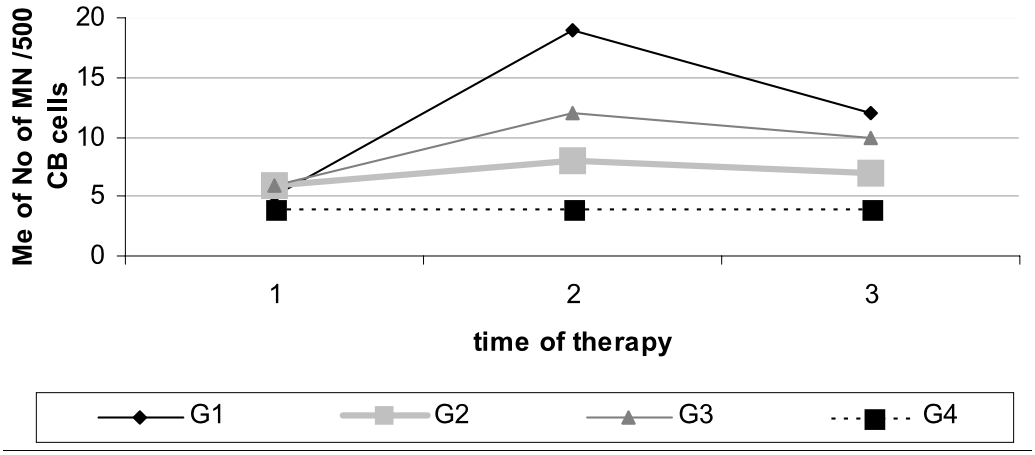

Figure 5. Median value of the MN of patients with testicular tumour (p = 0.005).

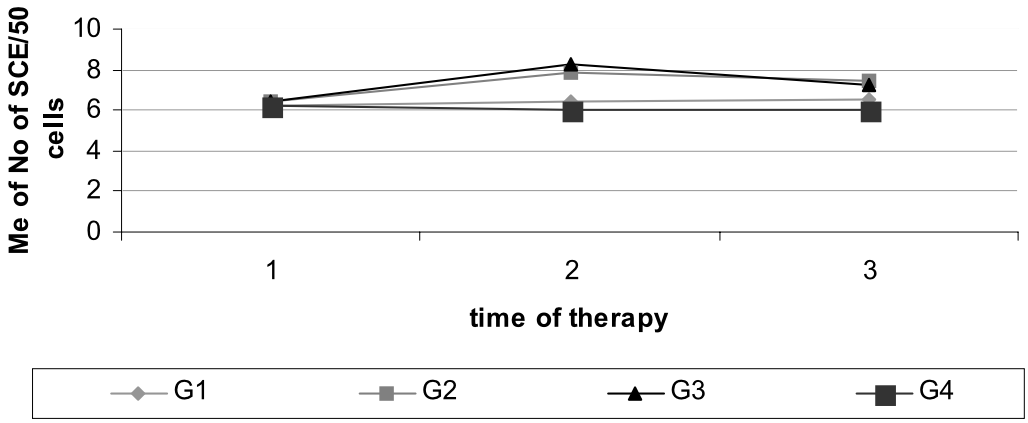

Figure 6. Median value of the SCE per cell of patients with testicular tumour (p = 0.001).

(p = 0.004). The comparison of numbers of chromosomal changes prior to the therapy and six months later was statistically significant in groups I to III (p = 0.001).

The number of micronuclei was also significantly higher in all the first three groups. It was the highest in the first group of patients treated by radiotherapy. Only in this group, the number of micronuclei was found to be significantly decreasing six month after the therapy (p = 0.001). Six months after therapy, MN values in the patients treated by radiotherapy and in those treated by chemotherapy according to BEPV schedule were significantly higher compared to pre-exposure levels.

Six months after chemotherapy, the number of SCE was still higher than the starting values (p = 0.001).

Discussion

Prior to treatment, we did not detect any deviations neither in the genome picture of our patients nor of the subjects of the control group without malignant disease.[21] The changes inside the genome were observed after chemotherapy as well as after radiotherapy.[22,23] From cytologic and mutagenetic points of view radiotherapy is more aggressive than chemotherapy, mostly because of a much higher level of unstable chromosomal changes, such as dicentrics. In irradiation, the mitotic activity in lymphocytes is significantly, yet only temporarily inhibited, whereas in chemotherapy, the inhibition of mitotic activity in vitro depends on the chemotherapeutic treatment scheme.

In a certain lapse of time after treatment, the genome picture is repaired. The time of repair depends on the length of exposure of the tissue-cellular complex and the type of damage. The genome is repaired because the damaged cells have died away. A great number of unstable aberrations, such as dicentrics, predominate in the patients treated by radiotherapy. These unstable cells are so severely damaged after radiotherapy that they are unable to survive and die shortly. In irradiated patients, a fast repair of the genome is mainly due to the following two reasons: the first is that a small tissue-cellular complex responds to radiation, and the second is that the structural chromosome damages predominate, especially unstable aberrations, such as dicentrics. The differences in the genome of individual cells are still possible. They may induce the development of oncogene and, consequently, of sec-

ondary tumour due to primary tumour treatment.[24]

The analysis of the treatment results in patients treated by chemotherapy showed that the difference between the chemotherapy according to BEPV schedule and the chemotherapy with paraplatinum was very small. In our patients, the only difference between both chemotherapy schedules was the number of micronuclei. This difference was statistically significant at all parameters in comparison to the first group been treated only by radiotherapy or the fourth group treated only by surgery.

Similar results were published also in other studies reporting of significantly higher effect of irradiation on the genome than that of chemotherapy [25,26] and of significantly higher effect of chemotherapy on the genome in comparison to the genome of the patients treated by surgery alone.[27]

Six months after the completed therapy, the number of cytogenetic changes was lower and not statistically significant any more. Monochemotherapy with paraplatinum is a new method of adjunct treatment of patients with an early stage of seminoma and is just being introduced at our Institute. The patients included in this study are the first to be treated with this method in Slovenia.[28,29] The advantages of paraplatinum monotherapy, lie primarily in short hospitalisation time, thereby also in shorter overall treatment time, as well as short-lived and mild side effects of chemotherapy,[3,4] as was also confirmed in our study.

In conclusion, the following question remains: Can the chromosome changes after chemotherapy, such as chromosome and chromatide breaks that take a longer time for the genome repair, also induce the development of oncogene and, consequently, of secondary tumours, considering that the cells with that kind of damage have more possibilities for surviving?

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