Cytogenetic monitoring of pharmaceutical staff working with cytostatic drug preparations: A 5-year follow-up

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Introduction. Cytostatic drug preparation in hospital pharmacies leads to a concentration of exposure on a few people and requires considerable protective measures.

Methods. To prove the efficiency of those standards, 45 people who routinely prepare cytostatic drugs were monitored with cytogenetic tests, Micronucleus-rate (MN), and Sister Chromatid Exchange-rate (SCE), once a year over a period up to 5 years. All participants of the study received a physical examination before they began preparing cytostatic drugs. Baseline MN and SCE were performed at the time. Individual comparisons for the tests between pre- and post-exposure, and a comparison between an exposed and an unexposed group were then performed.

Results. During the 5-year follow-up there were no hints of genotoxic effects as a result of handling cytostatic drugs.

Conclusion. Thus, it appeared that the safety precautions employed at this Institute were adequate.

Key Words: Sister chromatid exchanges (SCE); micronucleus (MN); cytostatic drugs; cytogenetic monitoring; pharmaceutical staff.

Cytostatic drugs are potent antineoplastic agents and most of them are hazardous chemicals with mutagenic, carcinogenic, and/or teratogenic properties.[1-3] Occupational exposure to these agents is therefore a concern for healthcare workers.

The target of all occupational safety precautions therefore is to avoid any exposure when handling these drugs, which need considerable protective measures, such as the use of special vertical laminar air-flow biohazard cabinets and suitable gloves to protect skin contamination. On the other hand, without well trained and experienced staff specialized in cytostatic drug preparations these safety precautions would almost have no effect.

For these reasons, cytostatic drug preparation centralisation in hospital pharmacies is considered necessary by both pharmacists and other medical disciplines. While limiting drug-handling to a restricted group of trained and experienced people can be advantageous; there is the possibility for higher potential drug exposure for these people due to higher throughput preparation.

Drug absorption during preparation as measured by direct cytostatic drugs biomonitoring in the urine of the pharmaceutical staff, has been shown in several studies.[4,5] Even using these safety standards, cytostatic drug absorption could be demonstrated, although the measured drug amounts were usually very small.[6,7] A recently published study stated that dermal absorption by permeation of cytostatic drugs like cyclophosphamide and 5-fluorouracil could be an important exposure route, even if latex gloves are used.[8]

On the other hand, the main risk for occupational exposed persons handling cytostatic drugs consists in genotoxic effects like chromosomal damage. Several epidemiologic studies in different countries could demonstrate those genotoxic effects especially under poor safety conditions.[9-15] The most often used cytogenetic methods in these studies were chromosome analysis (CA), sister chromatid exchange rate (SCE), and micronucleus test (MN).

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Table 1. Number of Persons, Age, and Occupation for Sex

	Total	female	male
Total	45	39	6
Age groups			
20–<30	24	21	3
30–<40	10	8	2
40–<50	9	8	1
50–<60	2	–	2
Occupation			
Pharm tech assistant	29	28	1
Chemist	9	4	5
Chemist's assistant	1	1	–
Nurse	6	6	–

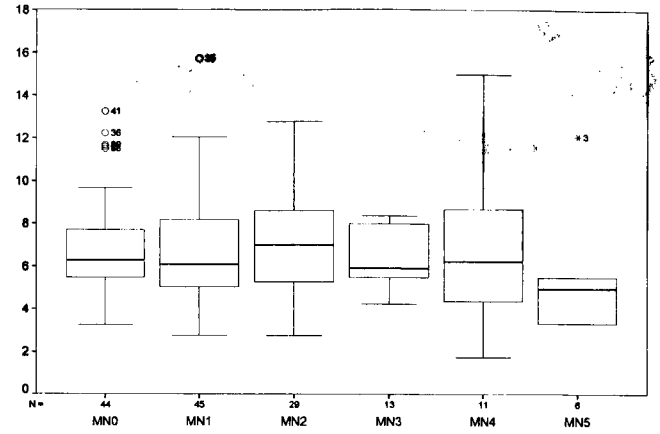

Figure 2. MN-Rate, six time points of measurement.

SUBJECTS AND METHODS

Subjects

Since the introduction of centralized cytostatic drug preparation in the pharmacies of some municipal hospitals in 1989 in Hamburg, the pharmaceutical staff (pharmaceutical assistants, pharmacists) were monitored using the cytogenetic test systems SCE and MN. We collected data from 45 people who had at least one cytogenetic test performed after exposure to cytotoxic drugs. A subcohort of 29 persons were monitored over a 2-year period with three cytogenetic tests; six people in this group were monitored over a period of five years with six tests (five tests during the time of exposure). The personel data of the participants are presented in Table 1. Age ranges from 22 to 56 years (Mean: 32 years). Twenty-six participants work in hospital pharmacies, and 19 work in outpatient departments. Six municipal hospitals in Hamburg were represented.

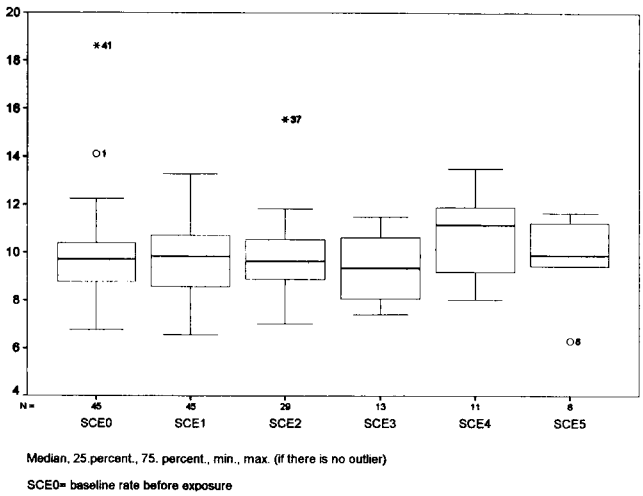

Figure 1. SCE-Rate, six time points of measurement.

An extensive initial examination was performed before beginning employment in order to get an individual baseline. In order to relate potential confounding factors with genotoxic effects, the initial history and physical examination included a detailed documentation of work conditions and personal data. Smoking habits, x-ray examinations in the past, past treatment, feeding habits (including alcohol consumption), and exposure to other genotoxic agents such as garden sprays, glues, solvents, and paints were recorded during this visit. The clinical examinations laboratory tests including hematological, immunological, renal, and hepatic parameters were performed yearly while participants were on study.

All subjects worked under high standards of safety conditions including a class II vertical laminar air-flow cabinet with fresh air supply. They used latex gloves which would be changed in any case of contamination and otherwise after a maximal use of one hour. Documentation of cytostatic drug preparation was recorded by a specific mass-oriented computer-based system, which allows the exact registration of the prepared agents and places quantitative data about possible exposition by counting possible preparation losses.

The time periods in the cytostatic drug preparation differed between the hospital pharmacies. In all affected hospitals, this duty is basically alternated with other functions in the pharmacy. Usually the personnel is preparing drugs the whole working day for one week and then rotates in another department of the pharmacy for a period of one to four weeks. The time of handling cytostatic drugs therefore varies from 20% up to 50% of the monthly working time.

While hospital personnel work under nearly stan-

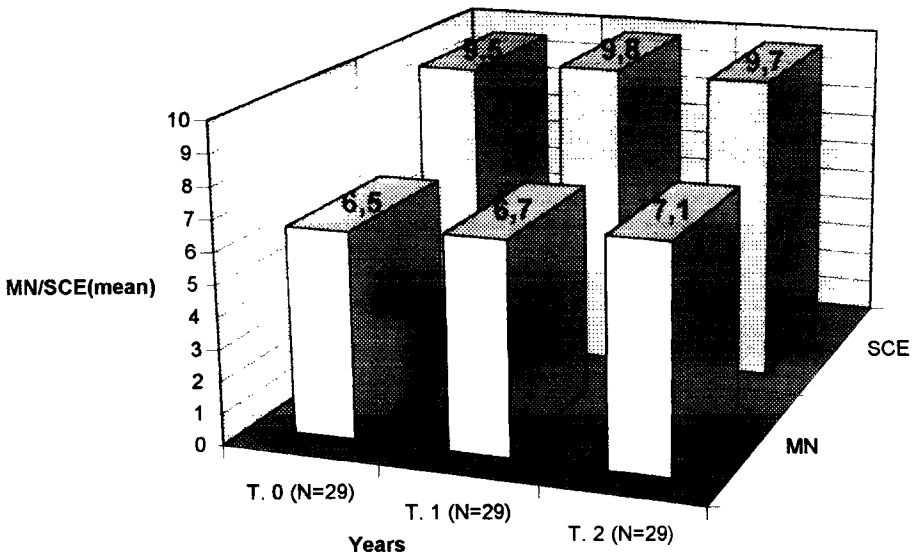

Figure 3. Cytogenetic tests (MN/SCE) from 29 persons over a period of 2 years.

dardized conditions, outpatient personnel work under varied conditions. Therefore it is nearly impossible to quantify cytostatic stress for this collective because they are exposed to the agent differently.

Cytogenetic examination

Cytogenetic examinations were made before the beginning and after every year of cytostatic drug handling. The examinations were carried out by the University Institute of Occupational Medicine (Prof Szadkowski) in Hamburg.

Cytokinesis-Blocked Micronucleus Assay (MN)

Five mL fresh whole blood is set up with 5 mL Ficoll Paque and mixed with standard cultural chromosomal medium. After resuspension, it is incubated for 44 hours (37°C). After addition of Cytochalasin B, incubate for a further 28 hours. Harvest after centrifugation, then swelling with hypotonic potassium chloride and, finally the preparation is fixed. Slides are made, airdried, and stained with DAPI. Two-thousand CB-blocked cells are scored. The results are given in MN/500 cells.

Sister chromatid exchanges (SCE)

Five mL fresh whole blood is set up with 5 mL under standard cultural chromosomal medium. After resuspension, 5 mL Bromdeoxyuridine is added. The mixture is cultured for 72 hours including 2 hours of colchicine (10 mg/mL) exposure. After harvest and fixation, preparations are stained with Acridine Orange and deep frozen over 12 hours. Metaphase plates with at least 40 centromeres are analysed, 15 metaphases were scored. The results are given in SCE/metaphase.

Statistical methods

SCE and MN frequencies were compared using the Mann Whitney Rank Sum-Test for unmatched data, and the Wilcoxon Test for pair differences, respectively. Differences where P values $<.05$ were considered significant.

RESULTS

The distribution of the SCE-rate and MN-rate are presented in form of box- and whisker-plots in Figs 1 and 2. There were no significant differences for SCE-rate or MN-rate between baseline data and the after exposure data.

No increased occurrences of SCE and MN were noted in the subcohort of 29 people with a monitoring period of two years under exposure as well (Fig 3). For

Table 2. SCE-Rate, Six Time Points of Measurement for 6 Persons

Case	SCE0	SCE1	SCE2	SCE3	SCE4	SCE5
1	8.77	12.13	7.48	7.63	8.43	9.50
2	8.88	11.25	8.24	10.90	11.15	9.44
3	9.72	9.84	9.57	8.07	8.85	6.32
4	9.89	9.62	9.77	10.63	13.50	11.66
5	10.40	8.19	7.20	11.46	12.95	11.24
6	10.79	6.56	10.45	7.41	11.85	10.28

SCE0 = baseline rate before exposure

Table 3. MN-Rate, Six Time Points of Measurement for 6 Persons

Case	MN0	MN1	MN2	MN3	MN4	MN5
1	3.70	5.01	5.03	4.25	4.74	4.97
2	4.50	4.47	7.00	8.25	7.99	4.98
3	6.18	5.19	5.00	5.93	6.25	3.24
4	6.34	4.34	6.07	5.71	8.62	5.49
5	6.97	6.49	7.47	8.36	1.73	3.32
6	11.60	10.11	5.72	4.73	9.50	12.10

MN0 = baseline rate before exposure

the six people who had a follow-up time of 5 years, the individual values are shown in Tables 2 and 3. There were no trends for increases in MN or SCE during this exposure time.

Our analysis does not show any significant differences for SCE and MN within the 5-year exposure period. This is particularly obvious for the homogeneous group of central preparing pharmaceutical assistants as demonstrated for hospital A (Fig 4).

This statement could also be confirmed by analysing the mean SCE and MN rate for three time points of measurements of persons working in hospital pharmacies and those working in outpatient departments as shown in Fig 5.

A significant correlation ($r = 0.32$; $P < .05$) was seen between MN and age, an observation which is in agreement with other study groups.[16] On the other hand we could not see a significant effect on SCE rate for smoking. In addition the statistical analysis of other possible confounders like x-ray, alcohol consumption, and remedy therapy showed no influence on the cytogenetic parameters.

DISCUSSION

Handling cytostatic drugs in hospitals requires a high standard of safety precautions to protect personnel from possible genotoxic hazards. The use of cytogenetic biomonitoring methods as a part of occupational health examination offers the chance to prove the efficiency of the safety standard, if those methods are sensitive to detect chromosomal damage. As cytostatic drugs can induce genetic damage by many mechanisms, cytogenetic methods should be evaluated taking this into account. Considering that chromosome analysis (CA) is too complex for routine use and methodological difficulties restrict the reliability of results, the MN-rate is an alternative test with several advantages. However, MN only analyzes acentric fragments of the spectrum of clastogen effects, but is still of value because of easy handling and the possibility to score a high number of interphase cells.[17] Moreover, mitotic defects can be detected.

Micronuclei represent long term effects of mutagenesis and correlate therefore with age,[16,18] an observation which could be confirmed by our data. Therefore we combined the MN rate with the SCE rate as a short-term test,[19] which has been shown to be a sensitive procedure to detect genotoxic effects of chemicals,[20] and cytostatic drugs.[21] Even if the exact mechanism of the SCE origin is unknown, this test can probably detect injuries either in DNA-synthesis or replication.[22]

Meanwhile several various studies regarding the

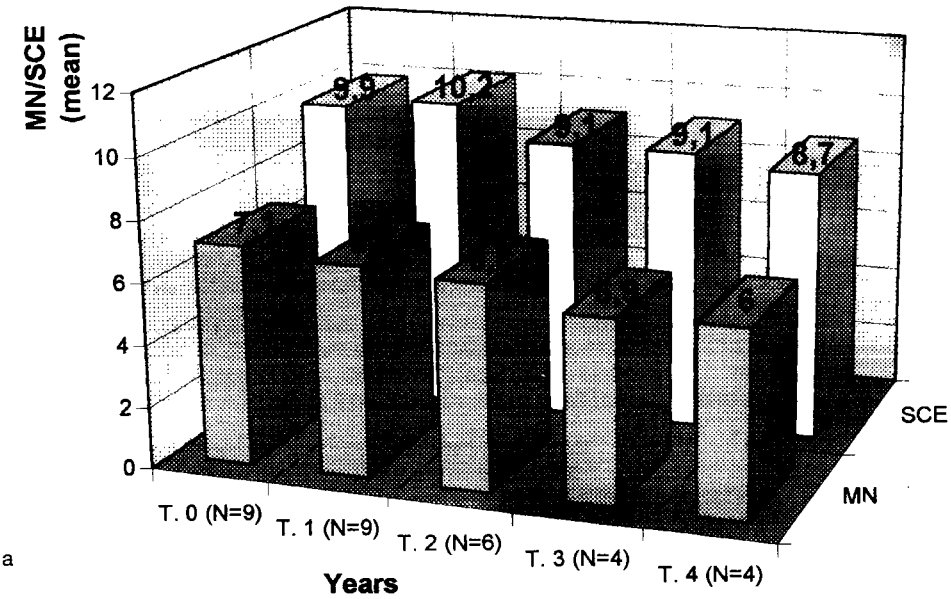

Figure 4. Cytogenetic tests (MN/SCE) over a period of 4 years in Hospital A.

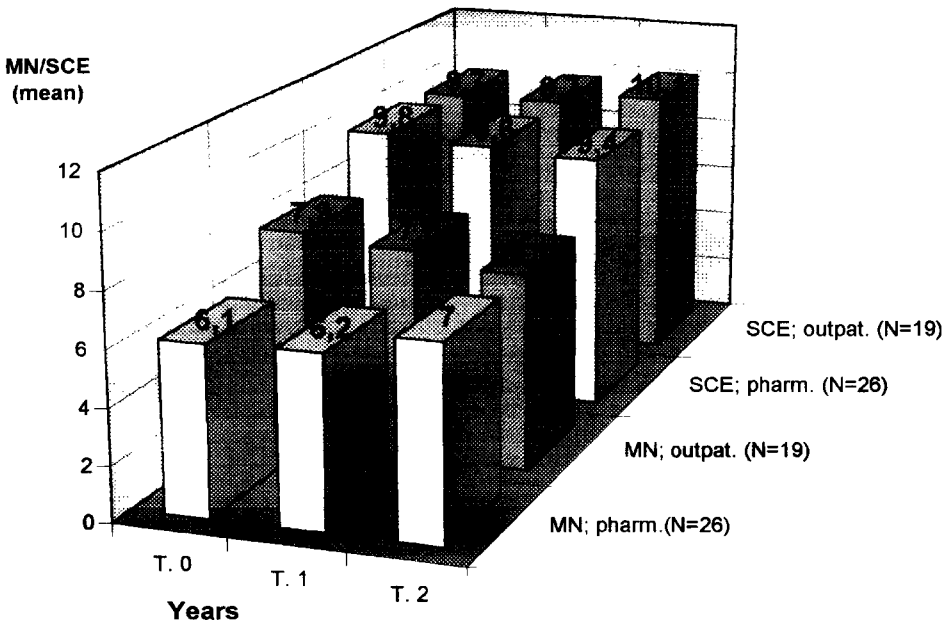

Figure 5. Cytogenetic tests (SCE/MN) in hospital pharmacies versus outpatient departments over a period of 2 years.

reaction of different cytostatic drugs related to SCE-rate and MN-Test were presented and show that genotoxic effects of most of the common agents can be evaluated at least with one of these test systems (Table 4).

Unfortunately all cytogenetic test-systems show a wide range of interindividual variability and therefore it is not possible to define standard scores for micronuclei frequence or for the sister chromatid exchange rate. The reasons for this interindividual variability could include the influence of non-occupational confounders like smoking, feeding habits, and recreational activities, or that there is predetermined genetic differences in mutagenic activity. Even in our study we saw remarkable interindividuals and intraindividual at various points of time. Therefore, we decided to choose a study design that allows a comparison between exposed and unexposed groups by observing the same people before and after exposure conditions. Moreover a longitudinal study with an observation period of 5 years enables the detection of long-term effects.

It should be noticed that there are no direct correlations between chromosome breakage and cancer risk in the same population which would be necessary to quantify risks to pharmacy and oncology health care personnel. On the other hand, an absence of genotoxic effects, as we could show in our study for the investigated pharmacy and oncology health care staff, is an important argument to support that the safety precautions employed protected these personnel against any hazardous effects by handling cytostatic drugs.

Table 4. Review of Cytostatic Drug-Induced Effects in MN and SCE

Cytostatic Drug	SCE	MN	References
Adriamycin	+	+	2; 25; 26
Azathioprine	−	+	26
Bleomycin	−	+	23; 26
Carboplatin	+	0	27
Cisplatin	+	+	2; 28
Cyclophosphamide	+	+	2; 18
Etoposide	+	0	24
5-Fluorouracil	−	+	2; 26
Methotrexate	+	+	2
Mitomycin C	0	+	26
Vinblastine	0	+	2; 26
Vincristine	−	+	2; 26

Note: (+) cytostatic drug induced effect documentated; (−) no effect observed; (0) no study found.

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were employed by us to improve the safety precautions when preparing cytostatic drugs for more than 5 years.

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