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Flow-Cytophotometric Studies on Urine Sediments of Patients Treated with Anti-Cancer-Drugs

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Summary. Urine specimens of 24 patients receiving polychemotherapy for malignancies of the gonads were examined by flow cytophotometry (FCM) and routine cytology. The results show abnormal DNA histograms during chemotherapy due to an arrest of the cell cycle at the S- and G_2M level. In treatment protocols with ifosfamide leucocyturia develops. No cytological changes of the urothelial cells occur during treatment. All these alterations are completely reversible within 4 weeks. It is concluded that the measurable nuclear changes of urothelial cells may serve as parameters for cellular events during polychemotherapy.

Key words: Polychemotherapy, Flow cytophotometry, Cytology, Cyclophosphamide, Cell cycle, Urothelial cells.

Introduction

Long-term survivors of childhood cancer treated by various chemotherapy protocols exhibit an increased risk for second malignancies [18, 19]. This is particularly high after treatment with cyclophosphamide, vinca alkaloids, or antifolic medications, but not with actinomycin D [10]. Also, patients treated with cyclophosphamide for a prolonged period of time show an increased risk of developing urinary bladder cancer [1, 11, 13, 27, 32]. The urine of patients receiving chemotherapy exhibited increased mutagenic properties [12]. Even the urine of nurses who handle cytotoxic drugs showed positive mutagenicity tests, so that definite short-term effects of those drugs on the urothelium can be expected [12].

These results prompted us to investigate various urinary parameters indicative of cell changes or other morphological effects in patients who received polychemotherapy courses.

Materials and Methods

We examined 23 patients with non-seminiferous testicular cancer (age from 19 to 42 years) and one case of dysgerminoma of the ovary, all of whom underwent polychemotherapy. In 18 cases chemotherapy started approximately 4 weeks after retroperitoneal lymph-node dissection and 6 patients received chemotherapy prior to surgery. Treatment consisted of 3–4 courses, each of 5 days duration at monthly intervals. All patients had sterile urine cultures at the time of examination. 200 ml of fresh urine were collected one day before, on the first day and on the fourth day of each chemotherapy course. The relevant details of the chemotherapy modalities as well as the number of patients and measurements are listed in Table 1. All patients treated with ifosfamide received the uroprotector sodium 2-mercaptoethanesulfonate (Mesnum®)[1] [17]. Urine samples were fixed and processed for flow cytophotometric (FCM) DNA analysis as well as for routine cytology as previously described [24]. DNA histograms were analysed with regard to the ploidy of the measured stem lines as well as to the G_2M peaks of cell cycle; G_2M values greater than 10% were considered abnormal. Cytological characteristics were graded according to Papanicolaou. In the urine sediment prepared according to a standardisised procedure using a cytocentrifuge (Shandon Co.), leucocytes and macrophages were semiquantitatively assessed. In our system an average count of up to 15 leucocytes and/or macrophages/high power field at 300-fold magnification was considered normal.

Results

Semiquantitative assessment of the leucocytes and macrophages revealed a distinct increase of these cells in urine samples during treatment in 36/117 (30%) patients (Fig. 1). As evidenced in Table 2, this effect is mainly attributable to the treatment with ifosfamide. In the same way, during chemotherapy, the percentages of both (pseudo) aneuploide stem lines (Fig. 2) and G_2M peaks (Fig. 3) gradually increase. In 38 of 39 (97%) serial measurements, FCM DNA analysis of urine samples under cytostatic chemotherapy revealed abnormal histograms. In 72 of 107 (67%), cell aggregations in the G_2M phase were observed exceding the limit of 10%. As an example, DNA histograms of a series of measurements are presented showing euploidy with increasing G_2M peaks during treatment (Fig. 4). In 35 of 107 measurements (33%), aneuploide-like histograms were obtained, possibly as a

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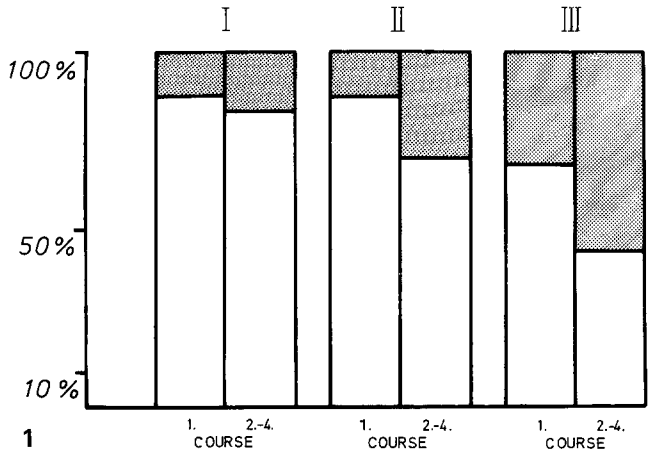

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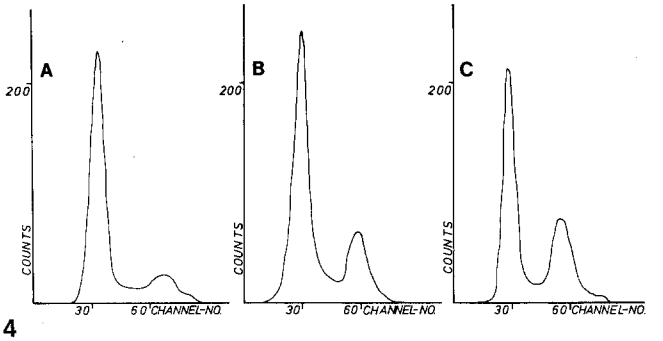

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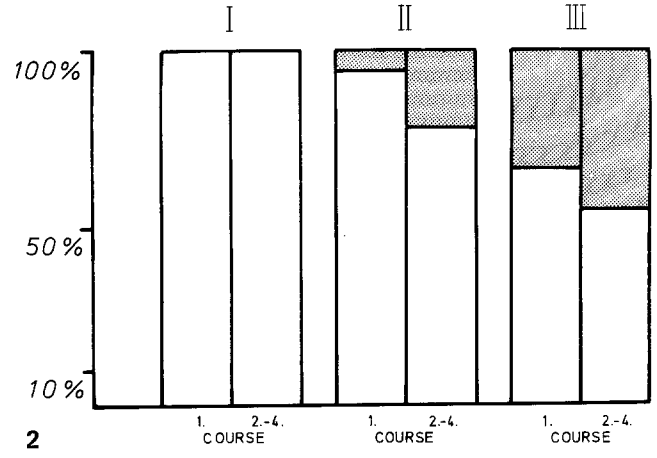

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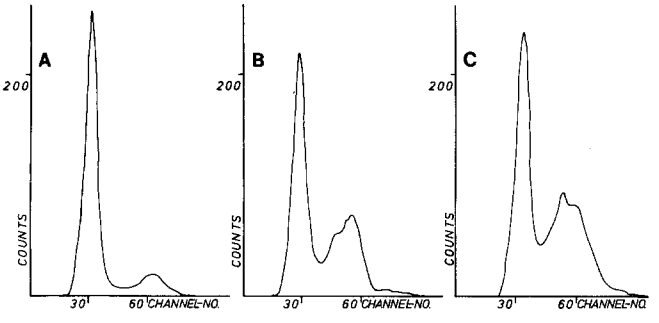

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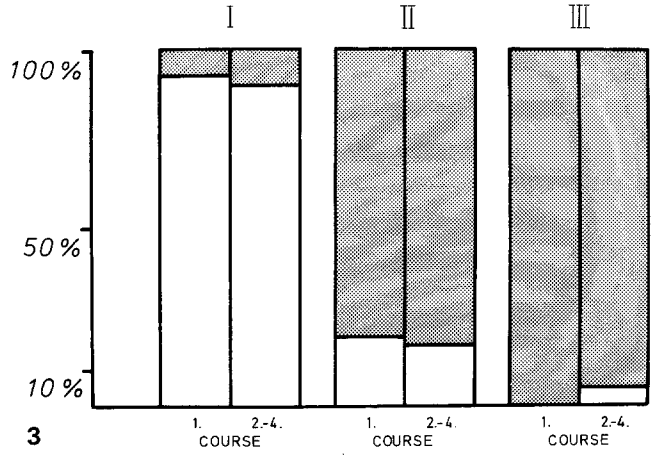

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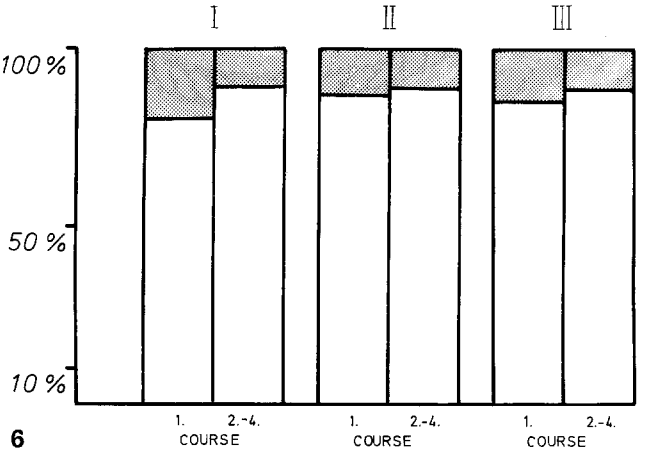

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Fig. 1. Percentage of patients with elevated leucocyte excretion with regard to the number of chemotherapy courses and the time of examination. ☐ = normal leucocyte excretions; ▨ = abnormal leucocyte excretion. **Figs. 1–3, 6:** *I* = one day before chemotherapy; *II* = first day of chemotherapy; *III* = fourth day of chemotherapy

Fig. 2. Percentage of patients with (pseudo) aneuploide DNA histograms with regard to the number of chemotherapy courses and the time of examination. ☐ = normal histograms; ▨ = (pseudo) aneuploide histograms

Fig. 3. Percentage of patients with elevated G_2M peaks with regard to the number of chemotherapy courses and the time of examination. ☐ = normal histograms; ▨ = elevated G_2M peaks

Fig. 4A–C. Example of euploide DNA histograms one day before (**A**), at the first day (**B**) and the fourth day of polychemotherapy (**C**) with increasing G_2M peaks

Fig. 5A–C. Example of (pseudo) aneuploide DNA histograms one day before (**A**), at the first day (**B**), and the fourth day of polychemotherapy (**C**)

Fig. 6. Percentage of patients with atypical cytological findings with regard to the number of chemotherapy courses and the time of examination. ☐ = normal cytology; ▨ = atypical cytology (Pap III)

Table 1. Modalities of the cytostatic chemotherapy, number of patients and treatment courses as well as the number of measurements. VBL = vinblastine (12 mg/m^2/2 days); BLM = bleomycine (60 mg/m^2/5 days); DDP = cis platinum (100 mg/m^2/5 days); IFO = ifosfamide (7,5 g/m^2/5 days); VP_{16} = eposide (500 mg/m^2/5 days)

drug combinations	No. of patients	No. of courses	No. of single-measurements	
			FCM	cytology/ leucocytes
VBL/BLM/DDP	15	23	65	69
VBL/BLM/DDP/IFO[a]	4	9	26	27
VP_{16}/DDP/IFO[a]	2	3	7	9
VP_{16}/IFO[a]	1	1	2	3
VP_{16}/BLM/DDP	2	3	7	9
total	24	39	107	117

[a] addition of sodium 2-mercaptoethane sulfonate (Mesnum), dosage according to Klein et al. [17]

Table 2. Percentage of increased leucocyte excretion in the urine with regard to cytostatic chemotherapy with or without inclusion of ifosfamide (IFO). *I* = one day before chemotherapy; *II* = first day of chemotherapy; *III* = fourth day of chemotherapy

chemotherapy	leucocyte excretion		
	I	II	III
without IFO	15%	16%	38%
with IFO	15%	38%	82%

result of an arrest in the S-phase. An example is given in Fig. 5. Further analysis of the treatment modalities does not reveal any relationship between administration of a drug, e.g. ifosfamide, and the percentage of abnormal DNA histograms. It is of particular intrest that the increased leucocyte excretion as well as the abnormal histograms at the end of each treatment course were completely reversible (Figs. 1–3).

In 102 of 117 (87%) examinations, routine cytology revealed normal nuclear differentiation (Pap I and Pap II) and in 15 cases (13%) atypical readings (Pap III). Atypias were equally distributed in urines taken before and during chemotherapy, so that no effect of the treatment could be found on urinary cytology (Fig. 6).

Discussion

The question of how and to what extent cytostatic drugs cause a damaging or any other effect to the urothelium deserves interest for several reasons.

Firstly, cyclophosphamide or its derivates are still widely used in polychemotherapy. Unfortunately, this drugs cause severe damage to the bladder urothelium with necrotisising cystitis, sometimes with a fatal outcome [23]. Therefore, the introduction of the uroprotector sodium 2-mercaptoethane surfonate represents a considerable success in this clinical situation [6, 9]. Today, administration of this substance to patients receiving ifosfamide is the usual way to prevent side effects on the urinary bladder. Despite this protection, in our series of 7 patients treated with this substance in combination with others, a significant leucocyturia during chemotherapy indicates a higher degree of damage to the urothelium in comparison with those patients treated without ifosfamide.

Secondly, many cytostatic drugs lead to an arrest of the cells in the S- or G_2M-phase, as proved by experimental [5, 20, 21] and clinical studies [2–4, 8, 15, 22]. As far as the generics used in our study are concerned, bleomycin [2, 4], alkylating agents, e.g. ifosfamide [4, 22] and VP_{16} [4] showed a pertuberation of the DNA histograms with a blockade of the S- or G_2M-phase of the cell cycle. No pertinent data exist on drugs related to alkyloids, e.g. vinblastine and cis-platinum. As a result of this arrest, an accumulation of cells in the S- or G_2M-phase is measured by different methods, among the most widely used ones being flow-cytophotometry [2, 3, 5, 7, 15, 16, 22, 25]. Our studies on urothelial cells both confirm and extend the data known from the literature. The cytostatic polychemotherapy used in our patient group is associated with a high percentage of elevated G_2M urothelial cells and a moderate portion of cells blocked in the S-phase. There is a clear time-dependent cell cycle arrest with accumulation of S- and G_2M-phase cells during the chemotherapy courses. Thus, from the standpoint of availability, urothelial cells seem to be very suitable to monitor the effect of cytostatic therapy in vivo. In the literature, a controversy is still unsettled as to the clinical significance of a blockade at the S- or G_2M level, respectively. Using FCM techniques, Smets et al. [22] were able to show a good correlation between cell cycle arrest and clinical response in patients with leukemia and lymphosarcoma. On the other hand, Büchner et al. [8] were unable to confirm these results in patients with the same disease. There is general agreement that no correlation can be established between the susceptibility of cells to a block of the cell cycle and the proliferative or vital capacity of the cells [4, 25]. In our opinion, FCM analysis of urothelial cells seems to be a very convenient, non-invasive method for monitoring the efficiency of chemotherapy on the cell cycle kinetics. Whether blocking of S- or G_2M phases correlates with the clinical effect of treatment remains to be determined for each tumour therapy protocol.

Thirdly, long-term cytostatic treatment, at least with cyclophosphamide, may be associated with a higher incidence of urinary bladder cancer [1, 11, 13, 27]. During a defined period of several chemotherapy courses, no such mutagenic effects can be expected, as evidenced by our urine cytology studies as well as by clinical experience. This point seems particularly important since in urinary bladder cancer FCM changes of the DNA histograms may exhibit exactly

the same abnormalities [14, 24, 26] as in our patients without urinary bladder cancer undergoing cytostatic treatment. The fact that abnormal DNA histograms after chemotherapy normalise within 3–4 weeks should be emphasised. At present, there is no other way to distinguish between these different conditions.

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