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DNA MEASUREMENTS AND CYTOMORPHOLOGY — A BASIS FOR PLANNING CHEMOTHERAPY IN ANAPLASTIC GIANT-CELL CARCINOMA OF THE THYROID

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Abstract — Drug induced changes in tumor cell population were monitored by cytophotometric DNA measurements and cytomorphological studies in 6 patients with anaplastic giant cell carcinomas of the thyroid. Cell samples were obtained by sequential thin-needle aspiration biopsies of tumors. The effect of intravenous Vinblastine infusion was tested in 6 and of Cisplatinum in 5 patients. Both drugs were active as judged by cytomorphological changes of tumor cells and DNA distribution patterns. Data obtained by monitoring drug induced changes were used for individualization of chemotherapy and multimodal treatment.

UDC: 616.441-006.6-076.5

Key words: thyroid neoplasms-drug therapy, giant cell tumors, DNA, photometry

Orig. sci. paper

Radiol. Iugosl. 21 (1) 47—53, 1987

Introduction — Anaplastic giant cell carcinoma of the thyroid is a rapidly fatal neoplasm with only few survivors over one year (1, 17, 19). Patients usually present with large inoperable tumors. The response to irradiation is rather poor and fails to control the disease in the neck. Various chemotherapeutic drugs were used in this disease with little success, with the exception of Adriamycine (5, 6, 13, 17, 19, 26). Recently, multimodal treatment seems to be promising for the improvement of local control (18, 29, 34) but there are still only few long term survivors (8, 9, 12, 17, 30, 31, 32, 33, 34).

Aims of the study: Combined chemotherapeutic schedules are to a great extent empirical, not taking into account: sensitivity of tumor cells in individual tumors and patients or the influence of a given chemotherapeutic agent on cell kinetics of a particular tumor. Based on our previous experience with squamous cell carcinomas (2) and sarcomas (3), we tried to induce changes in cell kinetics of anaplastic thyroid carcinomas by low doses of intravenous Vinblastine (VELBE) or Cisplatinum (CDP) infusion in order to enhance the effects of other drugs and/or irradiation. By monitoring the drug induced chages in tumor cell kinetics and morphology we intended to optimize the timing of the application of effector therapy. In addition, study of drug induced morphological changes in tumor cells could provide information on drug sensitivity in individual tumors.

Material and methods — Six patients (age 56—80, 4 females, 2 males) with anaplastic giant cell carcinoma were included in this study. Cell samples were taken from primary tumors in 4 patients and from metastatic deposits in 2 patients.

Cell samples were obtained by sequential thin-needle aspiration biopsies (ABC) of tumors before and at uneven intervals up to 96 hours after termination of chemotherapeutic drug infusions. Several smears were prepared from each sample, partly processed for DNA densitometric measurements and partly for morphological light microscopical studies. For DNA measurements smears were stained by Feulgen procedure including acid hydrolysis in 4 N HCl at 28° C for 60 minutes as described previously (2).

Received: October 27, 1986 — Accepted: December 13, 1986

DNA measurements were performed on a Vickers 85 scanning microdensitometer (condensor numerical aperture N. A. 1.3, objective N. A. 0.7) at wavelength 560 μm and processed by a computer. In each smear 150—250 tumor cells and 25—100 leukocytes were measured. The DNA value of leukocytes in the same smear served as a reference for diploid DNA value (L), the class interval in histograms was 0.25 L.

Cell morphology and cell distribution pattern were studied in smears air dried and stained according to May-Grünwald-Giemsa (MGG).

Chemotherapeutic drugs — dosage and schedules: Vinblastine sulphate (VELBE) 2 mg was infused intravenously over 12 hours in 2 patients and over 24 hours in 4 patients. Aspiration biopsies of tumors were performed up to 96 hours after the termination of VELBE infusion.

Cisplatinum (CDP) 50 mg/m² was administered in intravenous infusion over 8—24 hours (in 24-hour infusion in 2 patients, in 12-hour infusion in 2, and in 8-hour infusion in 1 patient). Aspiration biopsies were performed up to 24 hours after CDP.

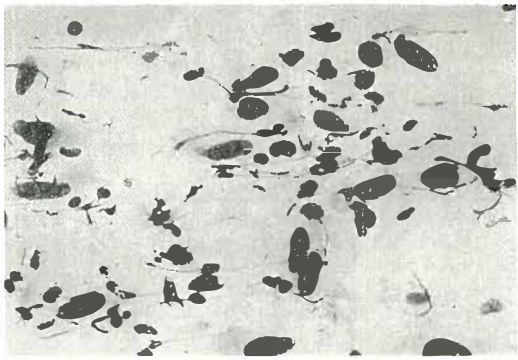

Fig. 1 — Anaplastic giant cell carcinoma — ABC specimen before treatment (patient P. F. No 6324/77) MGG, 25×4. Spindle-shaped tumor cell population

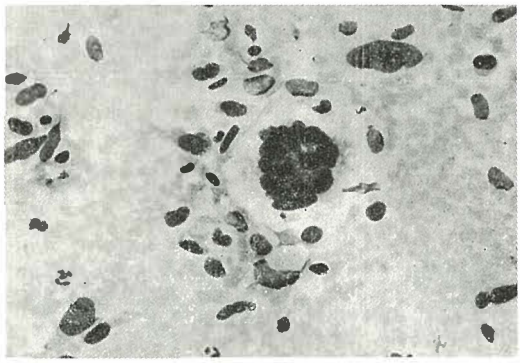

Fig. 2 — ABC sample of the same patient 23 hours after VELBE intravenous infusion 2 mg over 24 hours. MGG 25 × 4. Enlarged and pyknotic nuclei, multinucleated cell

Results — Cytomorphological studies: In slides stained according to MGG different evidence of drug influence on tumor cells was observed. Cellular, nuclear and nucleolar enlargement, multinucleation, degenerative changes in all cellular structures and nuclear pyknosis were found (see fig. 1, 2). Tumor cell population of the same sample can show all cited changes or only some of them. The time of onset and the severity of morphological changes after VELBE differed, thus apparently reflecting individual tumor sensitivity. The dependence of changes on the time of exposure to VELBE is also possible. This aspect deserves further study. Only in samples of two patients an increased number of cells in mitosis appeared after VELBE. The changes observed in ABC samples were not specific for a particular chemotherapeutic drug. Except for an increased number of cells in mitosis CDP caused similar morphological changes to VELBE. In addition to changes in cell morphology, altered cell adhesiveness resulting in an enhanced cell dissociation and loosening of cell clusters was observed.

Owing to a small number of observations after CDP it is not possible to draw any firm conclusion, although it seems that CDP infused over 24 hours resulted in a latter appearance of cell changes in comparison with 8- and 12-hour infusions.

DNA measurements: Due to a marked heterogeneity of anaplastic giant cell carcinoma demonstrated by a wide scatter of DNA values with several peaks in the histograms interpretations of drug induced changes in DNA distribution pattern in terms of cellular kinetics are very difficult. Nevertheless, after VELBE two types of changes were observed in the histograms: in 2 out of 6 patients there was an accumulation of cells with high DNA values

suggesting a transition delay and the end of S and G_2 and M phases (Fig. 3). In contrast to that, in 4 out of 6 patients, there was a reduction of cells with higher DNA values with a transition delay at the beginning of S phase (Fig. 4). This type of changes was observed after long infusions of

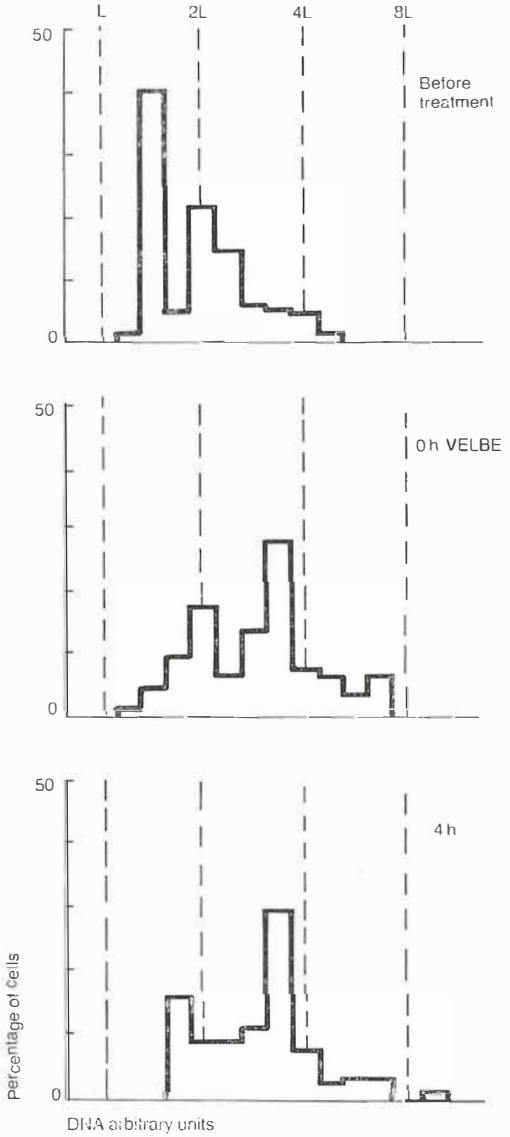

Fig. 3 — Anaplastic giant cell carcinoma (patient K. L. No 7368/84) DNA histogram before, 0 and 4 hours after the termination of VELBE infusion 2 mg over 12 hours. An accumulation of cells with high DNA values is demonstrated at 0 and 4 hours after VELBE

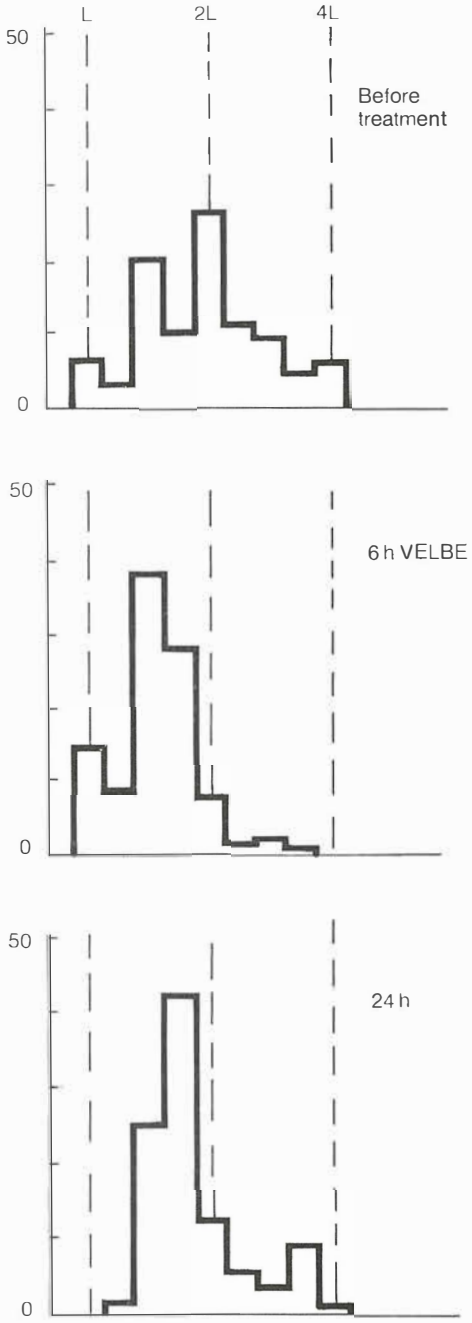

Fig. 4 — Anaplastic giant cell carcinoma — regional recurrence (patient K. A. No 2100/83) DNA histograms before, 6, 24 and 30 hours after the termination of intravenous VELBE infusion 2 mg over 24 hours. The number of cells with high DNA values is diminished after VELBE. The cells seem to be blocked at the beginning of S phase

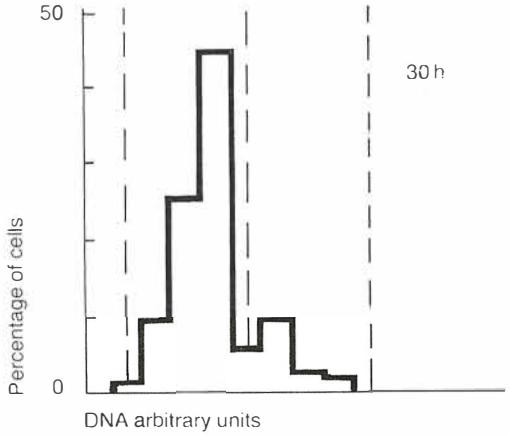

Fig. 4

VELBE (24 hours), whereas accumulation of cells with high DNA values was observed in both patients treated by 12-hour infusion of VELBE. There are not enough measurements performed so far to allow conclusions whether these two types of changes in the DNA distribution patterns are due to individual tumor sensitivity or a difference in the time of exposure to VELBE. The changes in DNA histograms of anaplastic thyroid carcinomas after CDP infusion were similar to those in squamous cell carcinomas, soft-tissue and bone sarcomas we studied in a previous work (2, 3). CDP seems to slow down the passage of cells through S phase with an accumulation of cells in late S and G_2 phases (Fig. 5). There was no increase in the number of mitoses found after CDP treatment.

The results of DNA studies and cytomorphology were used in planning therapeutic strategy in individual patients. The sensitivity to drugs was assessed by these methods. It was found that both VELBE and CDP are effective in anaplastic giant cell carcinoma. To our knowledge, there are no data on the effect of VELBE in this type of tumor. The data on changes in cellular kinetics induced by VELBE or CDP as re-

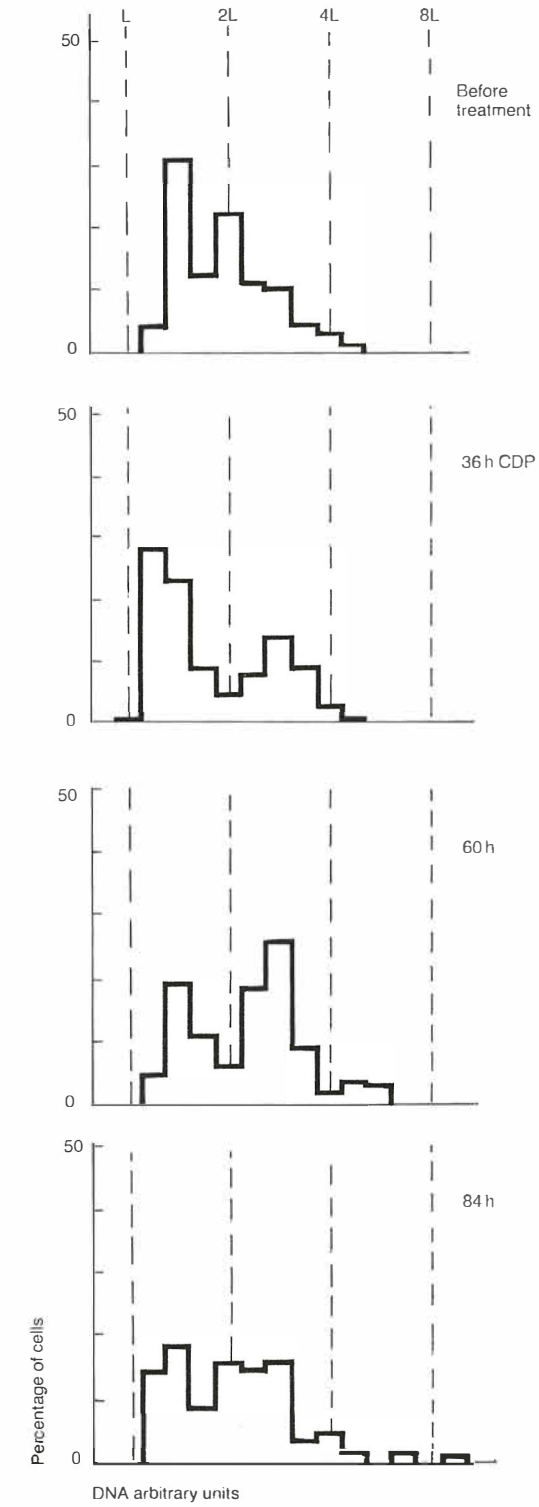

Fig. 5 — Anaplastic giant cell carcinoma (patient P. F. No 6324/77) DNA histograms before, 36, 60 and 84 hours after the termination of CDP infusion 50 mg/m² intravenously over 12 hours. An accumulation of cells in late S and G_2 seems to occur at 60 and 84 hours after CDP

flected in DNA histrograms were used in planning combined individualized chemotherapeutic schedules with promising clinical results. The intervals among drugs in schedules were individualized according to DNA measurements. Data from the literature on different phase sensitivity for damage by drugs were also taken into consideration while planning treatment (i. e. cells near S-G_2 boundary were reported to be most sensitive to VELBE) (10). The maximum killing effect by Bleomycin was obtained for G_2 cells (4, 14), CDP was found to be most toxic for G_1 gells (14). Cells in G_2 and M are most sensitive to irradiation (11, 22). These data were used together with the cytomorphological observations and DNA sequential histograms for planning of treatment in individual patient. For example: CDP infusion 50 mg/m^2 weekly and irradiation twice per day was planned in one patient taking advantage of long lasting accumulation of G_2 cells induced by CDP and demonstrated in DNA histograms in this patient. In another patient combined chemotherapy containing VELBE 2 mg, CDP 50 mg/m^2 Methotrexate 50 mg, Bleomycin 30 mg and 5-Fluorouracil 750 mg produced a dramatic response: a large soft tissue metastasis measuring 13 × 12 cm regressed completely already after the first course of treatment. In two other patients this schedule produced an important tumor regression more than 50 %.

Discussion — There are a few reports in the literature (7, 15, 20, 21, 25) on the cytokinetic effect of CDP in experimental tumors. To our knowledge, there was no such work reported on human solid tumors in vivo. In a previous work we studied the DNA patterns and cytomorphology after CDP in squamous cell carcinomas (2) and sarcomas (3) and used these data for planning of treatment with promising clinical results. There are several difficulties in interpretation of the results in such studies, one of them being the problem of sampling. ABC samples could differ considerably especially if they are taken from different parts of tumor and are not necessarily representative for the whole tumor. An additional drawback to the application of cytomorphology and DNA studies is heterogeneity of tumors. It is obvious that particulary in anaplastic tumors the results of DNA studies are difficult to interpret, and should be used with caution. A correlation of DNA distribution patterns with cytomorphological observations can yield useful data i. e. in the case shown in Fig. 3 enlargement of tumor cells, increased number of mitoses and multinucleated cells were found in MGG smears taken 0 and 4 hours after VELBE infusion. These data together with the DNA distribution pattern shown in Fig. 3 could indicate a block of cells in late S, G_2 and mitosis. Cytomorphological changes can be used only for a rough estimation of the cell damage. They are detected later than the changes in the DNA pattern. In addition, by cytomorphology alone we can get no information on the viability of cells showing only minimal morphological changes. Nevertheless, both DNA measurements and cytomorphological studies were of great help to the clinicians in planning and evaluating the effect of treatment.

Conclusions — 1. Vinblastine in low doses given in continuous infusion is an effective drug for anaplastic giant cell carcinoma.

2. DNA measurements and cytomorphological studies are useful in rational planning of chemotherapy and multimodal treatment.

Povzetek

Pri 6 pacientih z gigantocelularnim anaplastičnim karcinomom ščitnice smo proučevali spremembe v populaciji tumorskih celic s citofotometričnim merjenjem DNK ter s citomorfološkimi študijami.

Celične vzorce smo dobili s sekvenčno tankocelično aspiracijsko biopsijo. Delovanje intravenske infuzije Vinblastina smo proučevali pri 6, učinek Cisplatinuma pa pri 5 bolnikih. Učinkovitost obeh zdravil je bila vidna v citomorfoloških spremembah tumorskih celic ter v distribucijskem vzorcu DNK. Podatki, pridobljeni s spremljanjem sprememb, ki sta jih povzročili uporabljeni zdravili, so bili uporabni za individualizacijo kemoterapije in multimodalnega zdravljenja.

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