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CHEMOTHERAPY FOR HÜRTHLE CELL CARCINOMA BASED ON SEQUENTIAL DNA MEASUREMENTS***

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Abstract — Inoperable or disseminated Hürthle cell carcinoma is a therapeutic challenge as therapy with I^{131}, radiation or chemotherapy is usually ineffective. In order to find an effective chemotherapy the influence of Vinblastine (VLB) (2 mg bolus or infusion over 6, 12, 24^h) was studied in 5 patients (4 women, 1 man; age 43—69 years) and that of Cisplatinum (CDP) in 3 patients. CDP 20 mg was administred in two and CDP 50 mg/m^2 in one patient. Four patients had distant metastases, one locoregional disease only. Thin-needle aspiration biopsies of tumors (1 primary, 4 metastases) were performed before and repeatedly after VLB or CDP application. The smears were stained after Feulgen and were used for cytophotometric DNA measurements. VLB and CDP produced an increase of cells in S phase compartment. On the basis of changes produced in the DNA distribution pattern by the test dose of VLB or CDP chemotherapy was planned: either a sequence of 3 VLB infusions with individual intervals or a combination of VLB, Cisplatinum, Methotrexate, Bleomycin, 5-Fu and Adriamycin was used. All 5 patients responded — 1 CR, 3 PR, 1 MR. Chemotherapy was combined with surgery in one, and with radiation in 3 patients. Two out of 5 patients have NED 43 +, 29 + months after treatment, one patient is living with disease 41 months after chemotherapy, one patient died of tumor 5 months after chemotherapy and one patient 6 weeks after start of chemotherapy of pulmonary embolism with more than 90 necrosis in the tumor.

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Introduction — Hürthle cell carcinoma is a thyroid tumor of moderate aggressivenes (9). It is relatively uncommon (6, 8). An incidence from about 3%—10% of all thyroid carcinomas was reported (6, 9). It is considered to be a variant of follicular carcinoma by some authors (4, 11). The prognosis of Hürthle cell carcinoma, however, is much worse than that of follicular carcinoma (4). Surgery is the treatment of choice (8, 9, 12, 14). When inoperable or disseminated, Hürthle cell carcinoma is a difficult therapeutic problem as it usually does not concentrate I^{131} (6, 8, 9, 10, 12)

Author		No. of cases	Chemotherapy	Response
Thompson	1974 (14)	2	Endoxan	not cited
Gottlieb	1974 (7)	9	Adria	2/5 PR
Droz	1981 (5)	3	Adria VP-16, 5-Fu Endoxan	1/3 MR
Gundry	1983 (8)	9	Adria Various	∅
Miller	1983 (12)	1	CDP Adria	∅
Har-El	1986 (9)	4	not cited	3/4 ∅ 1/4 survived 5 yrs (CT + S + RTX)
Auersperg (present study)	1987	5	VLB sequence VLB, MTX, 5-Fu, Bleo Adra, CDP Individualized CT	1 CR, 3 PR, 1 MR

CT = chemotherapy
S = surgery
Rtx = radiotherapy

Table 1 — Results of chemotherapy in Hürthle cell carcinoma

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and is relatively radioresistant (6, 8). Har-El reported that paliation but never cure was achieved by radiation (9). Only few patients with Hürthle cell carcinoma were treated by chemotherapy (CT) (Table 1), therefore experience with this modality of treatment is scarce and most authors (5, 7, 8, 9, 12) consider the effect of chemotherapy in Hürthle cell carcinoma as disappointing. There are few reports on chemotherapy in thyroid malignancies — the schedule combining Adriamycin (Adria) and Cis-platinum (CDP) described by Shimaoka (13) is rather aggressive and therefore not easily applicalbe in patients with inoperable or disseminated Hürthle cell carcinoma. Those patients are usually in the older age group and have usually contraindications for agressive chemotherapy because of cardiovascular and other diseases.

In our previous studies sequential DNA measurements in aspiration biopsies of tumors together with morphological studies of changes in tumor cells, induced by chemotherapy proved to be useful in development of rational nonaggressive chemotherapy for a variety of rare or chemoresistant tumors (1, 2, 3). The aim of this study was to find an effective and nonaggressive chemotherapy for inoperable or disseminated Hürthle cell tumors which would be applicable in poor risk patients, or in patients after failure of standard chemotherapy for thyroid cancer, Adria and CDP (13).

Material and methods — From 1984—1987 5 patients (1 male, 4 females; age 43—69 years) with inoperable or disseminated Hürthle cell carcinomas in whom standard CT for thyroid cancer (Adria + CDP) was not possible, were treated with individualized chemotherapeutic schedules. Two patients were previously treated with CT; one with Adria and one with Adria + CDP. In 3 patients Adria and CDP in standard doses could not be given because of impaired renal function in 1, severe respiratory distress in 1 and cardiac arrhythmia in one patient.

The effect of low doses of VLB on tumor cell population 2 mg in bolus or i.v. infusion over 6, 12, 24^h was tested in 4 patients and that of CDP 20 mg i.v. over 2 hours in 2 patients, and CDP 50 mg/m^2 i.v. infusion over 12 hours in 1 patient. Thin needle aspiration biopsy of the primary tumor (1 patient) or metastases (4 patients) were performed before and at uneven intervals after VLB or CDP infusion. Half of smears were stained acc. May-Grünwald-Giemsa for morphological studies and the other half by a Feulgen

Patient No.	Tumor site	Chemotherapy	Other therapy	Follow-up
M. Z. (1) female 69 yrs	soft tissue metastasis right hip 16 × 12 cm	VLB CDP — PR VLB CDP Bleo 5-Fu MTX 2 courses — CR	radiation 5000 cGy	NED 43 + months
K. H. (2) female 56 yrs	locoregional tumor inoperable	VLB CDP VLB — PR CDP MTX Bleo — PR	∅	died 6 weeks after CT, pulmonary embolism, ~ 90 % necrosis of the tumor
J. B. (3) female 60 yrs	metastasis clavicle 15 × 11 cm	VLB CDP — PR VLB CDP VLB Bleo Adria Endoxan — PR	surgery	NED 29 + months
K. A. (4) male 60 yrs	Locoregional tu pulmonary metastases mediastinal lymph nodes	VLB VLB VLB — PR VLB Bleo MTX 5-Fu — PR At 21 months reinduction of CT VLB VLB VLB — PR	radiation + VLB 3000 cGy radiation 3000 cGy	alive with disease 41 months
G. M. (5) female 43 yrs	pulmonary metastases, mediastinal tu pleural, pericardial effusion, respiratory distress	VLB VLB VLB VLB Adria VLB CDP — MR 4.5 months	radiation 1000 cGy	dead 5 months

CT = chemotherapy

Table 2 — Patients characteristics

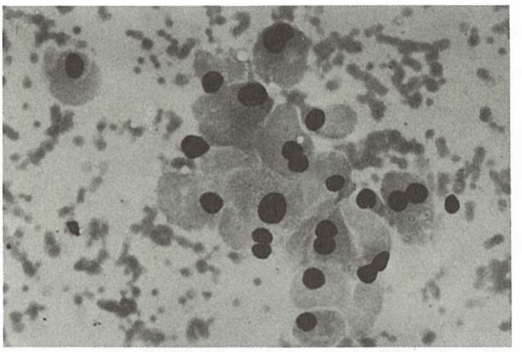

Fig. 1 — Patient No. 3, Hürthle-cell carcinoma, metastasis in the right clavicle.
Aspiration biopsy sample, Giemsa, 100 x.
Large, round and polyhedral cells: ample, homogenous, slightly eosinophylic stained cytoplasm, round, regular nuclei, fine chromatine structure, occasional nucleoli.

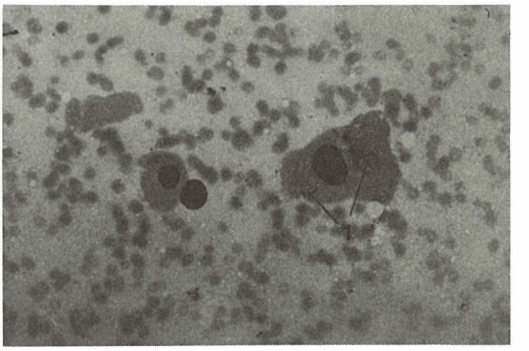

Fig. 2 — The same patient as in Fig. 1 54 hours after 2 mg VLB in 12-hour intravenous infusion, aspiration biopsy sample, Giemsa, 100 x. Few cells with slightly enlarged nuclei and vacuoles within the cytoplasm (1).

Patient No.		Chemotherapy	Response
(1)	test	VLB 2 mg 24^h interval 96^h CDP 70 mg 12^h	PR
	CT	VLB 2 mg 24^h interval 28^h CDP 70 mg 12^h interval 33^h MTX 50 mg 18^h Bleo 15 mg 8^h 5-Fu 500 mg 18^h 2 courses	CR
(2)	test	VLB 2 mg bolus VLB 2 mg 12^h	PR
	CT	CDP 20 mg 2^h interval 30^h VLB 2 mg 12^h Bleo 15 mg 12^h MTX 50 mg 16^h VLB 2 mg 12^h Bleo 15 mg 12^h	PR
			PR/90 % tu necrosis at autopsy
(3)	test	VLB 2 mg 12^h CDP 20 mg 2^h	PR
	CT	VLB 2 mg 12^h interval 120^h CDP 70 mg 7^h interval 10^h Bleo 15 mg 10^h Adria 40 mg 4^h Endoxan 400 mg 4^h VLB 2 mg 12^h Bleo 15 mg 12^h	PR
(4)	test	VLB 2 mg 12^h VLB 2 mg 6^h VLB 2 mg bolus	PR
	CT	VLB 2 mg 6^h Bleo 15 mg 6^h MTX 50 mg 12^h VLB 2 mg 12^h 1) 5-Fu 750 mg 12^h Bleo 15 mg 12^h 2) VLB 2 mg 12^h interval 36^h VLB 2 mg 12^h interval 36^h VLB 2 mg 12^h (test and CT 2 better than CT 1)	PR
(5)	CT	1) VLB 2 mg 12^h interval 24^h VLB 2 mg 12^h interval 18^h Adria 70 mg 7^h 2) CT 2 = 1 without Adria 3) CT 3 = 1	

CT = Chemotherapy

Table 3 — Chemotherapy

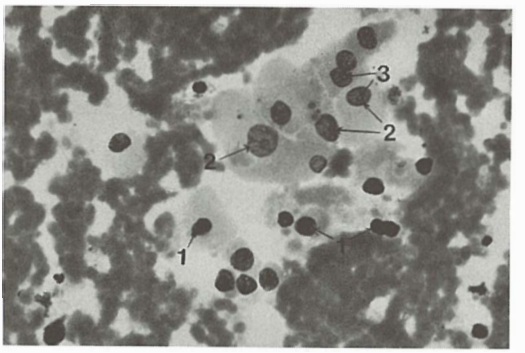

Fig. 3 — To same patient 72 hours after 2 mg VLB in 12-hour infusion. Aspiration biopsy sample, Giemsa, 100 x. Small picnotic (1) and hypochromatic, pale stained nuclei (2) and nucleoli (3).

staining procedure including acid hydrolysis in 4 NHCl at 28 °C for 60 min. DNA measurements were carried out on a Vickers 85 scanning microdensitometer at wavelenght 560 nm and processed by a computer. In each smear 150—250 tumor cells and 25—100 leukocytes were measured. The DNA value for leukocytes served as a reference value.

Data obtained by DNA measurement were used in planning of time sequence of cytostatics. It was attemped to infuse drugs predominantly effective in S-phase (MTX, VLB, Adria, Endoxan, 5-Fu) and G_2-phase (Bleo, VLB, Adria) during the time after VLB or CDP when DNA histograms showed a maximum in relative increase in S-and G_2-compartments.

A block of cells at the beginning of S-phase which was found after VLB was a basis for a combination of VLB and irradiation which was used in 2 patients after CT (patients No. 4 and 5, Table 2).

CT was individualized according to the results of DNA measurements and cytomorphological studies. Data on drugs, dosage and time sequence of cytostatics are in Table 3.

Results — Results of DNA studies — DNA distribution pattern after VLB was individual and seems to depend on the tumor sensitivity and time of exposure to the drug. In patients 1 and 4 (table 3) the number of cells with high DNA values was reduced after VLB. In patient No. 3 (Table 3) the cells in S-phase accumulated from 0—48^h, at later hours, the cells in were probably blocked at the beginning of S-phase (Fig. 5, A and B). In patient No. 2 a difference in the DNA distribution pattern depending on time of the exposure to the drug was demonstrated (Fig. 6). In

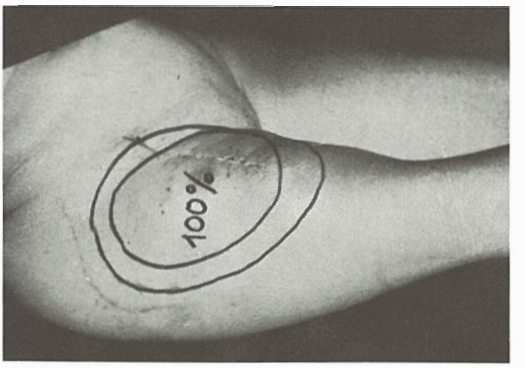

Fig. 4 — Patient No. 1, soft tissue metastasis of an anaplastic clear cell carcinoma (12 × 16 cm) before CT (outer circle), after test dose of VLB 2 mg/24^h and CDP 70 mg/12^h (inner circle). A CR was achieved after the first course of CT with VLB 2 mg, CDP 70 mg, MTX 50 mg, Bleo 15 mg and 5-Fu 500 mg.

patient No. 1 (Table 3) there was an impressive tumor reduction already after the test dose of VLB. The DNA histograms showed, however, only minor changes — endoreduplication — and later on elimination of cells with high DNA values.

After CDP 20 mg in 2^h there was an accumulation of cells in S- and G_2 + M compartment (Fig. 5 B) in patient No. 1 (Table 3) again — after CDP 70 mg in 12 hours — the cells with high DNA values were eliminated. The tumor in this patient regressed rapidly — 20 hours after the first chemotherapy no more malignant cells were obtained by aspiration biopsy.

Results of morphological study — Morphological changes of tumor cells after chemotherapy were found in aspiration biopsies of tumors in patients No. 1—4. In patient No. 5 the cell sample was inadequate. Different degenerative changes were caused by chemotherapy. The intensity of changes and the time of their onset seems to depend on the tumor sensitivity and the time of exposure to the drug. In the patients No. 2 and 4 the changes of tumor cells were more pronounced and were detected earlier after VLB 2 mg in 12^h infusion in comparison with VLB 2 mg in bolus. In patient No. 3 the first morphological changes were observed 54 hours after VLB 2 mg in 12 hours administration. Among morphologically unchanged tumor cells, cells with slightly

enlarged nuclei with pycnotic or regular chromatin structure and vacuoles within the cytoplasm could be found. More diffuse, however discrete changes appeared 72 hours after VLB administration; hypochromatic, pale stained nuclei, wrinkled nuclear membrane, pale stained nucleoli, degenerated cells and naked nuclei.

On general, the morphological changes appeared rather late 54—72 hours after chemotherapy and were rather discrete. In patient No. 4 (Table 3) the morphological changes appeared only 5 months after chemotherapy.

Clinical results — Four out of 5 patients responded, one patient had complete response (CR), 3 patients partial response (PR), one patient had minimal response (MR) and subjective improvement lasting 4, 5 months. This patient died 5 months after CT in respiratory distress. One patient died 6 weeks after CT of pulmonary embolism. At autopsy more than 90 % of the tumor was necrotic. Two patients are alive with no evidence of disease (NED) 29 and 43 months after therapy, one is living with disease 41 months after CT. CT was combined with surgery in one patient and with irradiation in 3 patients — the duration of the effect of CT alone could be evaluated only in patient No. 4 (Table 3). The PR obtained after test dose of VLB and 1st combined CT lasted 20 months. After the 1st CT this patient refused further treatment and had reinduction of chemotherapy only after tumor progression 21 months later.

Chemotherapy was well tolerated with no major side effects.

The patient No. 1 had malaise and loss of appetite during CT. Patients No. 2 and 3 had palpitations during infusion of VLB. Patient No. 2 developed diarrhea on month after CT. The fatal pulmonary embolism two weeks later in this case could be connected with dehydration.

Discussion — DNA studies in aspiration biopsies of Hürthle cell tumors yielded useful information for planning of CT. On the basis of changes in the DNA distribution pattern after VLB an effective regimen consisting of sequence of VLB infusions with individualized intervals between infusions was designed. The second and third infusion of VLB were applied during the time of accumulation of cells in S-phases. Another possibility was sequence of VLB and CDP

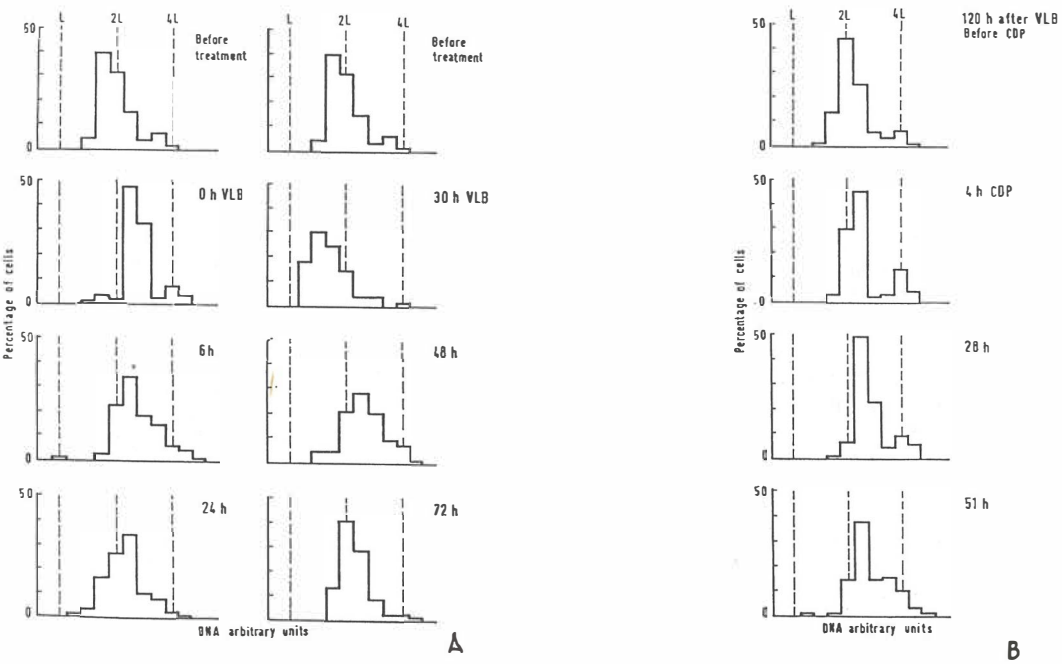

Fig. 5 — Patient No. 3, well differantiated Hürthle-cell carcinoma — metastasis in the clavicle. DNA histograms of histograms of aspiration biopsies specimens before, 0, 6, 24, 30, 48 and 72 hours after VLB 2 mg in 12-hour intravenous infusion (A). The DNA distribution pattern shows a progression delay through the S phase. The relative number of cells in S phase is increased from 0—48h, at 72—120 hours (B) the cells are probably blocked at the beginning of S phase. At 30h there is a shift of DNA values toward left. After CDP 20 mg in 2h infusion (B) there is a block of cells in the S phase and G_2 + M compartment.

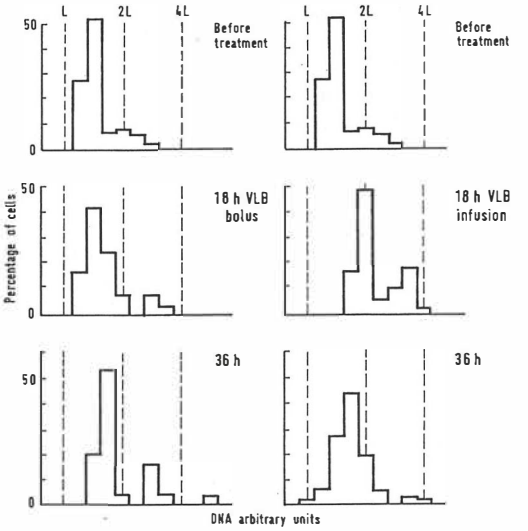

Fig. 6 — Patient No. 2, low differentiated Hürthle-cell carcinoma — inoperable primary tumor. DNA histograms of aspiration biopsies specimens before, 18 and 36 hours after VLB 2 mg in bouls and VLB 2 mg in 12-hour intravenous infusion. The DNA distribution patterns are different at 18 hours: After VLB in bolus there is an accumulation of cells at the beginning of S phase, whereas after VLB infusion the cells accumulate in the middle of S and $G_2 + M$ compartment.

infusion — CDP beeing applied during the block of tumor cells in the beginning of S-phase. When tested in the same patient, sequence of VLB or VLB and CDP infusions seemed to be more effective than combined chemotherapy with more drugs. The most impressive clinical response was achieved in patient No. 1 (Table 3). In this patient the first chemotherapy course resulted in a CR. The DNA histograms of the tumor in this patient, however, showed only minor changes. Apparently, the cells remained in a frozen state untill they were eliminated. Another interesting observation is the rather slow tumor regression and late onset of morphological changes after chemotherapy in patient No. 4, with long lasting effect of CT.

DNA measurements were an useful aid in planning CT. There is, however, not enough experience yet to assess the predictive value of changes in the DNA distribution pattern of tumors for the outcome of CT.

Conclusions — Low doses of VLB or CDP are effective modulators of cellular kinetics in Hürthle cell tumors. Cytophotometric DNA measurements were useful in designing non agressive effective chemotherapeutic schedules. A sequence of low doses of VLB and CDP infusion was effective in Hürthle cell carcinoma.
DNA distribution patterns of tumors after VLB infusion and bolus are different.

Povzetek

KEMOTERAPIJA PRI KARCINOMU HÜRTHLOVIH CELIC. PLANIRANJE NA OSNOVI MERITEV DNK

Zdravljenje inoperabilnega ali diseminiranega karcinoma Hürthlovih celic je težko, ker je zdravljenje z $J,^{131}$ obsevanjem ali kemoterapijo največkrat neuspešno. Z namenom, da bi izdelali uspešno in neagresivno kemoterapijo smo preučevali učinek nizkih doz Vinblastina (VLB) in Cisplatinuma (CDP) na karcinom Hürthlovih celic pri 5 bolnikih. Zasledovali smo razporeditev vrednosti dezoksiribonukleinske kisline (DNK) v vzorcih aspiracijskih biopsij tumorjev (barvanje po Feulgenu, meritve na posameznih celicah) pred in po infuziji VLB in CDP ter zasledovali učinek VLB in CDP na morfologijo tumorskih celic. Po VLB in CDP smo ugotavljali relativno kopičenje celic v S fazah celičnega ciklusa, kar smo izkoristili za načrtovanje kemoterapije. Uporabili smo infuzijo 3 zaporednih odmerkov VLB z individualnimi presledki ali pa kombinacijo VLB, CDP, Methotrexata, Bleomycina, 5-Fluorouracila in Adriamycina. Pri vseh 5 bolnikih smo dosegli zmanjšanje tumorja (1 popolen regres, 3 delni regresi, 1 minimalen regres. Kemoterapijo smo kombinirali z operacijo pri 1 bolniku in z obsevanjem pri 3 bolnikih. Dva bolnika sta brez bolezni 43 in 29 mesecev, 1 živi z boleznijo 41 mesecev. Dve bolnici sta umrli, ena 5 mesecev, druga 6 tednov po začetku kemoterapije. Ta bolnica je umrla zaradi pljučne embolije, v tumorju ščitnice smo našli 90 % nekroze po kemoterapiji.

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