Urological Research 7, 249-251 (1979)

Urological Research

DNA Content in Cells Aspirated from Carcinoma of the Prostate Treated with Oestrogenic Compounds*

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Accepted: August 21, 1978

Summary. Prostatic transrectal fine needle aspiration biopsy and flow cytometric analysis of the single cell DNA content was carried out in 8 patients with untreated carcinoma of the prostate before and at frequent intervals during treatment with oestrogenic compounds. Before treatment, the undifferentiated tumours were characterised by hyperploid cell populations while diploid cells were found in well differentiated cases. During treatment a change towards diploid DNA distributions was observed in hyperploid cancers between 3 and 6 months after initiation of therapy. Supplementary flow cytometric DNA analysis may be of value in follow-up studies of patients with prostatic cancers particularly of more poorly differentiated types.

Key words: DNA- Prostatic carcinoma - Oestrogenic treatment.

In a previous investigation flow cytometry (FCM) and transrectal fine needle biopsy were used to obtain DNA histograms in patients with benign prostatic hyperplasia and prostatic carcinoma. It was observed that increasing anaplasia paralleled an increasing occurrence of hyperploid cells (1). Sprenger et al. (5) observed diploid cells in a case of carcinoma of the prostate treated with oestrogen. Kjaer et al. (4) found diploid DNA content in 16 out of 18 patients with long-term treated carcinomas of the prostate. Diploid cells in the prostatic aspirates paralleled a good clinical course. This could correspond with the fact that only prostatic carcinomas with stem lines mostly in the diploid area respond to hormone therapy or that a good response to hormone therapy is in harmony with complete disappearance of tumour cell stem lines with high ploidity.

The purpose of this prospective study was to investigate the DNA distributions in cells from patients with prostatic carcinoma during hormone treatment.

MATERIAL AND METHOD

12 patients entered the study (average age 72 years, range 56-85 years).

4 patients had to be excluded for various reasons, 2 died within two months, therapy had to be withdrawn due to cardiac failure in one and one patient was only followed for 4 months.

The treatment compounds used were oestradiol phosphate and oestramustine phosphate.

The patients underwent fine needle aspiration biopsy of the prostate as described by Franzén (3). The aspirates were used for cytomorphological evaluation and FCM analysis.

The procedures were carried out prior to therapy and at short intervals during treatment, viz. after one day, one week and then monthly throughout the observation periods which ranged from 9 to 21 months.

FCM analysis was carried out as follows: The aspirated material was washed once in TRIS EDTA buffer. After centrifugation the cells were stained according to the detergent technique described by Vindeløv (6). The cells were suspended,

*Read in part at the meeting in Danish Urological Association, Copenhagen, April 1978.

0300-5623/79/0007/0249/$01.00

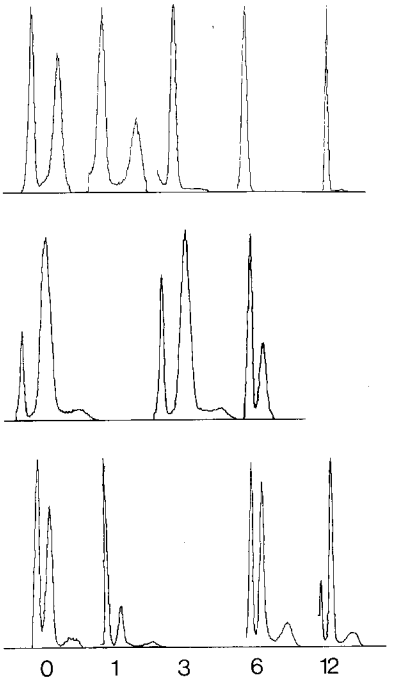

Fig. 1

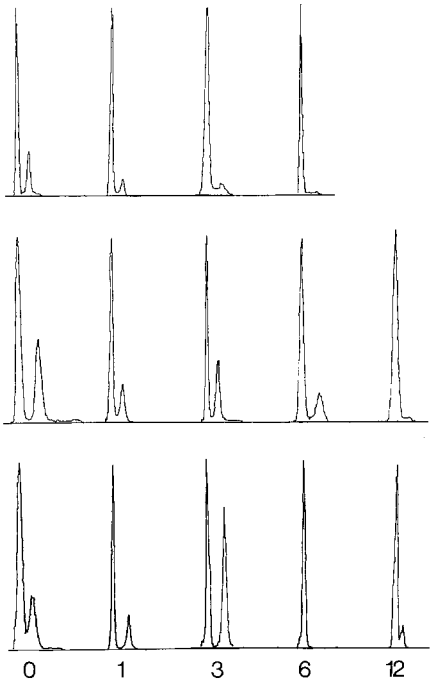

Fig. 2

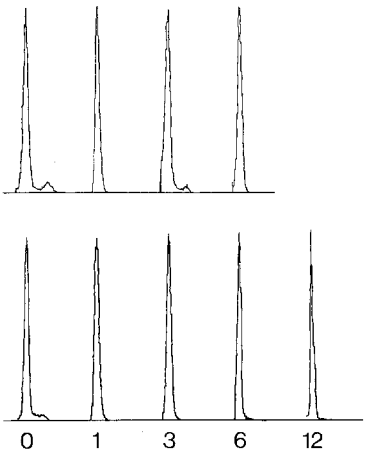

Fig. 3

Figs. 1-3. DNA histograms based on the analysis of about 50.000 cell nuclei per histogram. Ordinate indicates cell/channel in relative units and abscissa the relative fluorescence intensity. One peak indicates all counted cell nuclei in the diploid area. Two or three peaks represent hyperploid cells. Time intervals are given below in months from initiation of treatment

Fig. 1. Histograms from three patients having poorly differentiated carcinomas. In one patient in the upper row a reduction of initial 2 major peaks to a single peak is apparent from month 3. Patients in middle and lower row did not respond to treatment, although some variations can be noted in the hyperploid cells. Fig. 2. Histograms from three patients having moderately differentiated carcinomas. All patients end up with a single peak. The patient in the lower row withdrew from therapy after 2 months with a resulting increase in the hyperploid peak (month 3). After resuming treatment the hyperploid peak disappeared (months 6 and 12). Fig. 3. Histograms from two patients having well-differentiated cancers. An insignificant second peak can be noted before treatment (month 0). This is however an equally frequent finding in benign lesions

stained and kept in a solution containing ethidium-bromide and RNase. After filtration this method results in a suspension of single nuclei, the purity of which was controlled under microscope. After staining for at least 10 minutes the nuclei were analysed in a flow microfluorometer (Cytofluorograph, Biophysics Inc.). The signals were sorted and stored in a DIDAC 800 (Intertechnique) multichannel analyser and the histograms were presented as a plot of the number of measured cells against the relative fluorescence intensity (or relative DNA content) on a chart recorder. The calculation of the percentage distribution of the various cell populations was performed automatically by integration in the DIDAC 800. In each case between 12, 000 and 100, 000 nuclei, with an average of 50, 000 were analysed.

RESULTS

The resulting histograms are given in Figures 1-3. Six patients initially having poorly and moderately differentiated cancers (Figs. 1 and 2) showed hyperploid cell populations in the histograms. Similar DNA profiles were obtained after one day, one week and as a rule up to 2-3 months after onset of therapy. At that time a change to continuing diploid DNA content was observed in four patients. A simultaneous change in cytomorphology from a dominance of cancer cells to a mixture of degenerated cancer cells and benign cells was noticed in these patients. In patients with initially diploid cancers (well differentiated on cytology) (Fig. 3) no apparent change in the histograms were observed although a good hormone effect was noted in the cytologic smears.

DISCUSSION

In this prospective series patients having prostatic carcinomas were followed closely during treatment with repeated fine needle biopsies and recording of the DNA content in the aspirated prostatic cells.

The diploid cancers responded well to treatment, but as a diploid DNA content is a normal finding in benign hyperplasia, prostatitis and some cancers (Bichel et al., (1),) this finding gives no additional information in follow-up studies. However, in four of six patients with initial hyperploid cancers a change to continuing diploid values was observed and this finding furthermore paralleled a good clinical response

Thus our previous findings of predominant diploid cells in successful long-term treated cancers (Kjaer et al. (4)) could be explained by a hormone effect both on diploid and hyperploid cancers.

Flow cytometric DNA analysis is therefore suggested as a useful supplement in the follow-up of hyperploid cancers of the prostate.

REFERENCES

1. Bichel, P., Frederiksen, P., Kjaer, T., Thommesen, P., Vindelφv, L. L.: Flow microfluorometry and transrectal fine-needle biopsy in the classification of human prostatic carcinoma. Cancer 40, 1206 (1977)
2. Esposti, P. L.: Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. Scandinavian Journal of Urology and Nephrology 5, 199 (1971)
3. Franzén, S., Giertz, G., Zajicek, J.: Cytological diagnosis of prostatic tumours by transrectal aspiration biopsy. A. Preliminary report. British Journal of Urology 32, 193 (1960)
4. Kjaer, T. B. et al.: Flow-cytometrisk DNA-analyse og cytomorfologisk vurdering af carcinoma prostatae i φstrogenbehandling. Ugeskrift for Laeger 140, 158 (1978)
5. Sprenger, E., Volk, L. und Michaelis, W. E.: Die Aussagekraft der Zellkern-DNS-Bestimmung bei der Diagnostik des Prostatakarzinoms. Beiträge zur Pathologie 153, 370 (1974)
6. Vindelφv, L. L.: Flow microfluorometric analysis of nuclear DNA in cells from solid tumours and cell suspension. Virchows Archiv B, Cell Pathology 24, 227 (1977)

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