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# VOLUME-WEIGHTED MEAN NUCLEAR VOLUME IN DYSPLASTIC GLANDS FROM THE VENTRAL PROSTATE OF RATS TREATED WITH CADMIUM CHLORIDE, AND RESEMBLING HUMAN PROSTATIC INTRAEPITHELIAL NEOPLASIA

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## ABSTRACT

Estimates of volume-weighted mean nuclear volume have been performed in dysplastic epithelium from ventral prostate of rats treated with chronic oral exposure to cadmium chloride. The nuclear size of dysplastic cells was significantly increased in comparison to both controls and treated non-dysplastic acini. The high variability detected in the lowest level of sampling for the measurements of dysplastic glands may be due to the important hetereogeneity of their nuclei, similar to that observed in human prostatic intraepithelial neoplasia. The estimation of nuclear $V_V$ could prove interesting to study the behaviour of both neoplastic and pre-neoplastic lesions in experimental carcinogenesis as it has been stated for human tumors.

**Keywords:** cadmium chloride, dysplastic glands, rats, ventral prostate, volume-weighted mean nuclear volume, human prostatic intraepithelial neoplasia.

## INTRODUCTION

The chronic oral exposure to cadmium chloride is an interesting model of experimental prostatic cancer induction in rats (Waalkes and Rhem, 1992). It has been suggested that the cadmium could have a significant role in human prostate carcinogenesis (Waalkes and Rhem, 1994). A positive correlation among large values of the volume-weighted mean nuclear volume (nuclear $V_V$) and the frequency of stromal invasion, mortality or a more aggressive behaviour has been ascertained for several types of tumors including cutaneous melanoma (Sørensen, 1989), transitional bladder cancer (Fuzukawa et al., 1995 ; Sørensen, 1992), breast carcinoma (Ladekarl, 1995) and squamous cell carcinoma of the lung (Ladekarl et al., 1995). The nuclear $V_V$ was also useful as a parameter related to prognosis in human prostate cancer (Fujikawa et al., 1995). The present study is directed to ascertain the interest of this nuclear

size parameter in the study of the biologic behaviour of the pre-neoplastic lesions induced by cadmium in ventral prostate of the rat and also to establish the possible similarities between those lesions and those described in human prostate as prostatic intraepithelial neoplasia (PIN) (Bostwick, 1996).

## MATERIALS AND METHODS

A total of 15 adult male Wistar rats were used. Ten animals were exposed to cadmium chloride continually supplied in the drinking water (80 ppm) for 18 months. The 5 remaining rats were mantained as controls without cadmium. Dysplastic pre-invasive epithelial lesions, similar to human PIN, were described in the ventral prostate of 50% of the treated animals. Those animals and the controls were used for the stereologic evaluation of the nuclear $V_V$. The prostatic ventral glands were fixed in 4% paraformaldehyde for 24 hrs and vertical sections were used to allow unbiased measurements (Gundersen et al., 1988). Both control and experimental ventral prostate specimens were agar embedded and cut in 2mm width blocks, perpendicular to the sagital axis of the gland. Three of these blocks were systematically random sampled in each animal, and three slices per block were performed after rotating at random over the bench, around the vertical axis (perpendicular to the horizontal plane, i.e. the bench). Those were paraffin embedded and serially sectioned at 5μm. The estimation of the nuclear $V_V$ was carried out on three systematically random sampled hematoxylin-eosin-stained vertical sections per slice, using the stereologic software GRID (Interactivision, Silkeborg, Denmark) (Martín et al., 1997). This programme allows the selection of the fields to study per section by random systematic sampling after the input of an appropriate sampling fraction. In the present study, an average of 135 nuclei were point sampled per case, because the number of nuclei to be estimated per specimen in order to obtain reliable results is considered within the range of 70-100 (Artacho et al., 1992).

The sampling protocol for the dysplastic glands was sligthly different, because the dysplastic changes were multi-focally observed only in a few of the sections from experimental specimens: at least two histological sections containing dysplastic acini were chosen per specimen, then, each cluster of dysplastic acini was examined at low magnification, and systematically random sampled fields were selected for measuring. A number of fields from non-dysplastic acini lying among the dysplastic ones were also chosen for nuclear $V_V$ estimation, in order to compare the measurements of the dysplastic lesions with those of the histologically normal acini from the same animals. All the measurements were carried out using an Olympus microscope equipped with a 100× oil immersion lens (numerical aperture of 1.4) at a final magnification of 1200×. The average of individual measurements (nuclei) and fields investigated in each specimen is expressed in Table 1. The program used to evaluate the nuclear $V_V$ enables the generation of random test-lines directions that were superimposed onto the microscope images. The nuclear intercepts can be measured (Sørensen, 1991) along these test-lines. The lenght of nuclear intercepts ($l_0$) was processed to obtain $\pi\ l_0^3 / 3$, an unbiased estimate of nuclear $V_V$ independent of nuclear shape, which, because of point sampling, emphasizes larger nuclei rather than smaller ones. In addition, estimates of nuclear $V_V$ combine information about the three-dimisional nuclear size with knowledge of variability of nuclear size (Gundersen and Jensen, 1985). The measurements obtained in the treated animals were compared with those obtained in controls. In the group of animals with PIN-like lesions, the mean nuclear size measured in the dysplastic glands was also compared with the measurements obtained in the glands without histological changes.

All those comparisons were carried out by ANOVA using Fisher and Behren's test. The contribution of each of the three sampling levels (i.e., nuclear intercepts and their measurements, fields of vision, and individual cases) to the totally observed variance associated with estimates was investigated by nested analysis of variance (Gundersen and Osterby, 1981). The coefficient of error of measurements was also calculated by means of the following formula (Artacho et al., 1992):

$$CE\,(V_V) = [\,\Sigma\,(l_0^3)^2 / (\,\Sigma\, l_0^3)^2 - 1 / P_{nuc}\,]^{1/2} \qquad (1),$$

where: $l_0$ = lenght of nuclear intercepts; $P_{nuc}$ = points sampled.

## RESULTS

Dysplastic pre-invasive epithelial lesions have been observed in the ventral prostate of 5 treated animals (ranging between 12 and 18 months of exposure to cadmium chloride). The lesions had a multifocal distribution throughout the ventral prostate. Those glands showed an enlarged epithelial layer with occasional cribiform pattern. The nuclei were abundant and irregular in shape, evidenced by both the crowding and their increased size (Fig. 1).

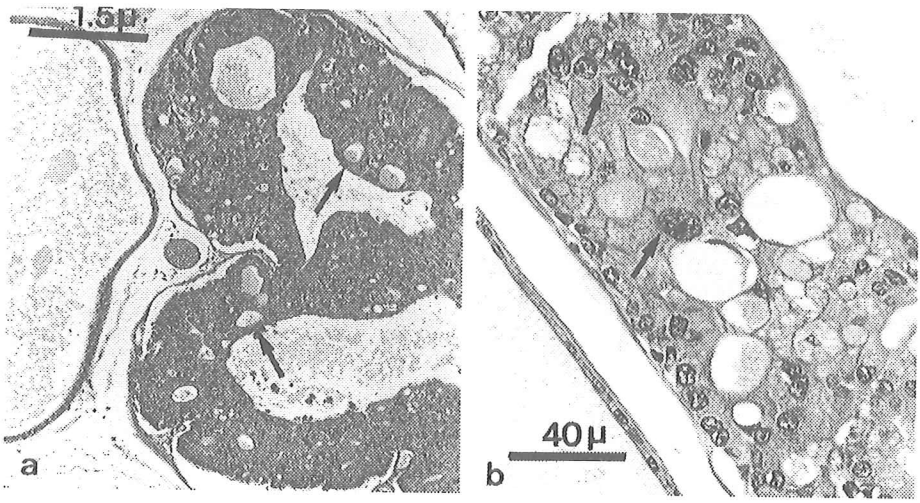


*Fig. 1 . (a) Dysplastic pre-invasive epithelial lesion observed in ventral prostate of a rat exposed for 18 months to cadmium chloride. The enlarged epithelium shows a cribiform pattern (arrows). (b) More detail from an area of an epithelial lesion showing remarkable variability in the size of the nuclei (arrows).*

The mean ± SD values of the nuclear $V_V$ in controls and treated rats : normal + dysplastic glands, normal glands only, and dysplastic glands only, are shown in Table 1 and Figure 2.

*Table 1. The relative contribution to overall variance is estimated by regarding the observed variance at each level of sampling as the sum of the true variance at that level plus the variance of the mean (SEM)$^2$, at the lower level of sampling. Thus, large variances at the lower levels are diminished in their contribution to totally observed variance by the number of observations at that particular level.*

| **Cases** (mean ±SD)* (n=5) | **sampling level** (av. # of nuclei or fields) | **observed variance** | **variance of the mean** | **estimated variance of the mean** | **contrib. to total var. (%)** |
|---|---|---|---|---|---|
| **controls** | measurements (108) | 5935 | 55 | 55 | 17.0 |
| 110[a]±18 | fields (44) | 3452 | 78.4 | 23.4 | 7.2 |
| | cases | 324 | 324 | 245.6 | 76.0 |
| **treated** | measurements (212) | 17442 | 82 | 82 | 28.3 |
| 158[b]±17 | fields (70) | 7879 | 112 | 30 | 10.3 |
| | cases | 289 | 289 | 177 | 61.4 |
| **normal acini** | measurements (75) | 8949 | 119 | 119 | 13.0 |
| 109[a]±30 | fields (31) | 4081 | 132 | 13 | 1.4 |
| | cases | 900 | 900 | 768 | 85.6 |
| **dysplastic acini** | measurements (140) | 19735 | 141 | 141 | 49.0 |
| | fields (39) | 7661 | 196 | 55 | 19.0 |
| 183[b]±17 | cases | 289 | 289 | 93 | 32.0 |

(*) Values with different superscripts are significantly different ($p<0.01$).

In both control and treated rats, the greatest contribution (76-86%) to the totally observed variance was provided by the highest level of sampling, i.e., by the biologic variation among the cases. However, when the group of dysplastic glands was considered, the contribution to the total variance provided by the nuclear intercepts and their measurements were higher (49%) than that provided by the individual cases (32%) (Table 1).

The average of the coefficient of error in both control and treated animals was 0.07.

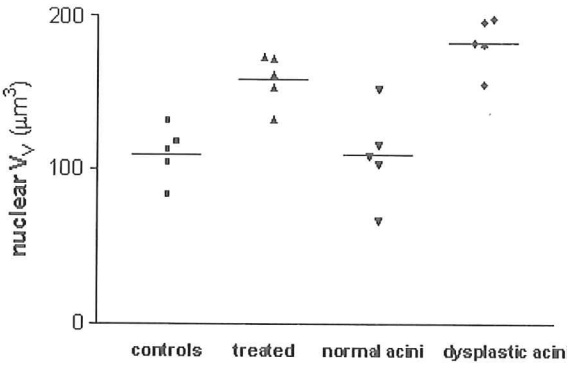


*Fig. 2. Scatter plot representing the individual nuclear $V_V$ from controls and treated rats. The horizontal lines in each column indicate the mean of each group.*

## DISCUSSION

The present study confirms the observation of other authors about the induction of dysplastic changes in rat ventral prostate by oral exposure to cadmium chloride (Waalkes and Rhem, 1994).

The results of the stereological measurements of the nuclear size -nuclear $V_V$- were significantly higher in the dysplastic epithelium from treated animals than in controls and normal glands from treated rats. These features might constitute a sign of evolution to malignancy as it was stated in the human prostate carcinoma (Fujikawa et al., 1995). On the other hand, the non-dysplastic glands did not show significant changes of nuclear size in comparison with controls.

The low values of the coefficient of error in both controls and treated animals are indicating a high efficiency of the method employed.

The high variability detected in the lowest level of sampling for the measurements of dysplastic glands may be due to the important hetereogeneity of their nuclei, similar to that observed in human PIN (Bostwick, 1996). The stereologic estimation of nuclear $V_V$ could be interesting to study since the behaviour of both neoplastic and pre-neoplastic lesions in experimental carcinogenesis has been reported for human tumors (Sørensen, 1992; Fukuzawa, et al., 1995; Ladekarl, 1995). The variation of nuclear size in these dysplastic changes could constitute a useful marker of the progression of prostate cancer that might correlate with the immunohistochemical and the molecular changes reported in the course of the evolution from benign epithelium to intraepithelial neoplastic changes (Bostwick, 1996).

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