Effects of combination endocrine treatment on normal prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma

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Abstract

Aims—To investigate the effect of combination endocrine treatment (CET) or luteinising hormone releasing hormone agonist and flutamide on non-neoplastic prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma.

Methods—The morphology, including the mitotic activity, of 12 radical prostatectomies from patients with prostatic adenocarcinoma pretreated for three months with CET was evaluated in haematoxylin and eosin stained sections and compared with an untreated age and stage matched control group.

Results—A differential effect on the non-neoplastic prostate was observed. In fact, the transition zone of the treated prostate showed simplification of the glandular lobules: the ducts and acini were small without undulations of the epithelial border and with a prominent basal cell layer. Within the peripheral zone there was inconspicuous branching of the ducts and acini which looked dilated and lined by flattened atrophic epithelium. Prostatic intraepithelial neoplasia occurred in scattered ducts and acini in the peripheral zone of 10 of the 12 patients. The epithelial cell lining showed a prominent basal cell layer. A certain degree of secretory cell type stratification was always present. However, crowding was less evident than in the untreated prostate because of cytoplasmic clearing and enlargement as a result of coalescence of vacuoles. The treated adenocarcinomas had neoplastic acini which looked small and shrunken, and areas of individual infiltrating tumour cells separated by abundant interglandular connective tissue. The secretory cells of the non-neoplastic, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma lesions had inconspicuous nucleoli, nuclear shrinkage, chromatin condensation, and cytoplasmic clearing. Apoptotic bodies were easily identifiable in all the cell layers. The lumina were rich in macrophages, sloughed secretory cells with degenerative features, and apoptotic bodies. Mitoses were not observed in any of the treated non-neoplastic prostate, prostatic intraepithelial neoplasia, or prostatic adenocarcinomas, whereas the mitotic frequency increased from non-neoplastic prostate through prostatic intraepithelial neoplasia up to prostatic

adenocarcinomas in the untreated specimens.

Conclusions—CET before radical prostatectomy causes regressive epithelial changes together with enhanced apoptosis and blocked mitotic activity.

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For many years, testicular androgen deprivation by oestrogen treatment or orchidectomy has been the standard hormonal treatment for advanced invasive adenocarcinoma of the prostate.¹ Recently, new drugs, especially agonists of luteinising hormone releasing hormone (LHRH) and anti-androgens, such as flutamide, have been introduced.^{2,3} Although the efficacy of these new drugs used alone is equivalent to that of standard treatment, their use avoids the major side effects of oestrogen treatment and the psychological impact of orchidectomy.⁴ Treatment combining an LHRH agonist and a pure antiandrogen drug (combination endocrine treatment or CET) to block simultaneously adrenal and testicular androgen has been shown to be superior to either approach used alone.⁵⁻⁷

Some histological changes induced by CET in human prostate cancer have recently been described.⁸⁻¹¹ However, there is scant information on the differential effect of an LHRH agonist and flutamide on the normal prostate zones and, in particular, the influence of CET on prostatic intraepithelial neoplasia.

The aim of our study was to evaluate the effect of CET on non-neoplastic prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinomas in resected prostatectomy specimens with emphasis on morphology and mitotic activity.

Methods

The study included the histopathological material from 12 consecutive patients (age range 60 to 70 years) who had undergone a radical prostatectomy for stage B prostatic adenocarcinoma¹² after temporary CET. Before surgery the patients had received CET for three months. Radical prostatectomy was performed while the patient was still receiving treatment.

The histopathological material of 60 cases (20 of benign prostatic hyperplasia, 20 of prostatic intraepithelial neoplasia, and 20 of prostatic adenocarcinoma) included in a previous publication¹³ was used to compare the

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histological changes induced by CET with the prostate morphology of untreated specimens. The control group (age matched with the treated group) underwent a radical prostatectomy for stage B prostatic adenocarcinoma without receiving chemotherapy, hormone, or radiation treatment before surgery. We chose to use a separate control group of untreated patients instead of the pretreatment prostate biopsy specimens because the latter type of diagnostic procedure does not usually yield representative material containing prostatic adenocarcinoma, prostatic intraepithelial neoplasia, and non-neoplastic prostate for a thoroughly comparative study, especially for mitosis counts.

The prostatectomy specimens were delivered fresh from the operating room shortly after excision. They were cut into slices about 0.3 cm thick and then fixed for 24–48 hours in neutral buffered formalin (4%), dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin wax. The haematoxylin and eosin stained sections of the radical prostatectomies of the treated group were reviewed by one of us (RM) to select slides containing prostatic adenocarcinoma, prostatic intraepithelial neoplasia (if present), and non-neoplastic tissue. Slides with acute and chronic inflammation were discarded. The slides selected for prostatic adenocarcinoma also covered the peripheral zone of the prostate including the capsule—that is, the advancing front of the carcinoma where the proliferative activity is thought to be higher than in the centre of the tumour nodule.⁹ Because the prostatic epithelium adjacent to prostatic intraepithelial neoplasia and prostatic adenocarcinoma usually shows a proliferative pattern,¹⁴ the sections selected to represent the non-neoplastic tissue were at a distance from prostatic intraepithelial neoplasia and prostatic adenocarcinoma and covered the peripheral and transition zones. The former comprises about 70% of the mass of the glandular prostate and is adjacent to the rectum. The latter surrounds the urethra from the base of the gland tapering to the verumontanum.

Mitoses were assessed on conventional 3 μ m thick histological sections (microtome setting) cut from formalin fixed, paraffin wax embedded material. The research project was conducted using haematoxylin and eosin stained sections. For the analyses of mitotic frequency and location, the sections were evaluated by one of our team using a Leitz Orthoplan microscope equipped with a $\times 63$ objective and an eyepiece graticule.¹³ The criteria used to identify the mitotic figures and to distinguish them from apoptotic bodies have been detailed before.¹⁵ In particular, mitoses are characterised by the absence of nuclear membrane, absence of a clear zone at the centre, by the presence of hairy instead of triangular or spiky projections, and basophilia of the surrounding cytoplasm. In contrast, apoptotic bodies are round or oval in shape and variable in size, made up of masses of pyknotic chromatin surrounded by narrow

cytoplasmic rims. The percentage of mitoses from a minimum of 1000 nuclei per case (also called the mitotic index) was calculated separately for each cell layer. The time needed to analyse each case was about 60 minutes.

The data were stored in an Apple Macintosh II computer. StatView II software was used for the calculation of the mean and standard error of mean (SEM), as well as for statistical analyses (Kruskal-Wallis and Mann-Whitney U tests). Reproducibility was tested for by duplicate evaluations of mitoses in six untreated cases (two benign prostatic hyperplasia, two prostatic intraepithelial neoplasia, and two prostatic adenocarcinoma), but no significant differences were found.

Results

For the treated group, the following morphological patterns of prostate diseases were present and investigated in the 12 patients: non-neoplastic prostate ($n = 12$), prostatic intraepithelial neoplasia ($n = 10$), prostatic adenocarcinoma (seven cases with acinar pattern, four with cribriform pattern, and one with solid or trabecular pattern). For the untreated group, the following morphological patterns of prostate diseases were investigated in prostatectomy specimens: non-neoplastic prostate ($n = 20$), prostatic intraepithelial neoplasia of low grade (PIN_{low}) ($n = 10$), and prostatic intraepithelial neoplasia of high grade (PIN_{high}) ($n = 10$), and prostatic adenocarcinoma (five cases with cribriform pattern or PAC_{cri}, five with solid or trabecular or PAC_{solid}, five with small acinar or PAC_{smac}, and five with large acinar or PAC_{lgac}). The morphology of the untreated cases has already been extensively reported.^{14 16 17}

NON-NEOPLASTIC PROSTATE

Within the peripheral and transition zones of the untreated non-neoplastic prostate, ducts and acini were morphologically similar to simple rounded contours that were not perfectly circular because of prominent undulations of the epithelial border. Both ducts and acini were lined by columnar secretory cells containing pale cytoplasm and separated from the basement membrane by noticeably flattened cells having dark, slender, filiform nuclei, and usually little or no discernible cytoplasm (basal cell layer) (fig 1). Apoptotic bodies were very seldom seen. Mitoses were extremely rare (mean (SEM) value 0.001 (0.001)%) and seen exclusively in the basal cell layer, where their mean (SEM) value was 0.002 (0.003)%. No difference was observed between peripheral and transition zones. The lumina contained occasional macrophages and rarely apoptotic bodies.

The transition zone of the treated non-neoplastic prostate showed important architectural and epithelial cell changes which were never seen in the untreated sections. Simplification of the glandular lobules was the consistent major feature on all specimens at low magnification, although the lobular

Figure 1 Untreated non-neoplastic prostate (transition zone). The ducts and acini have simple rounded contours. They are lined by columnar secretory cells with pale cytoplasm and by a flattened basal cell layer.

Figure 2 The transition zone of the treated non-neoplastic prostate shows simplification of the glandular lobules. The ducts and acini are small, ovoid, round, or comma-shaped. A two cell type epithelium is recognisable.

configuration typical of the transition zone was retained. The ducts and acini were small (less than 0.1 mm in diameter, whereas in the untreated specimens the diameter was usually more than 0.15 mm), ovoid, round, or comma-shaped. There were no undulations of the epithelial border due to the presence of corrugations of the wall. A two cell type epithelium—that is, a basal and luminal (secretory) cell layer—was recognisable. The secretory cells had inconspicuous nucleoli, nuclear shrinkage, chromatin condensation, and cytoplasmic clearing. The basal cell layer was prominent (fig 2). Focal squamous and

transitional metaplasia as well as basal cell hyperplasia were seen. Within the peripheral zone there was inconspicuous branching of the ducts and acini which looked dilated, star-shaped, and lined by flattened atrophic epithelium, which, in general, was single-layered and seldom double-layered (fig 3). This pattern was diffuse and involved the entire peripheral zone in all the treated patients. In the untreated cases this atrophic change was predominantly subcapsular and involved only occasional glands, with segmental distribution. Atrophic and cystically dilated glands were rare in the transition zone in both untreated and treated patients. Neither squamous and transitional metaplasia nor basal cell hyperplasia were seen in the peripheral zone. Apoptotic bodies were seen more frequently than in the untreated prostate at the level of the epithelial cell layers as well as in the lumina. In the latter macrophages were also increased. No mitotic figures were present in the treated cases.

PROSTATIC INTRAEPITHELIAL NEOPLASIA

In the untreated cases prostatic intraepithelial neoplasia always occurred multifocally and was located generally in the peripheral zone. In PINlow, cell crowding was present and accompanied by irregular nuclear spacing as well as a prominent increase in nuclear size. In addition to the increased size variability, nuclei showed finely granular chromatin and a few albeit prominent nucleoli usually located centrally; the cytoplasm was in general moderately clear and was eosinophilic in some of the cells (fig 4). In PINhigh, the deviations from normal were more evident than in PINlow. In particular, the degree of cytological abnormality was comparable with that of adenocarcinoma: presence in most nuclei of large, prominent eosinophilic nucleoli positioned peripherally, chromatin margination, noticeable nuclear enlargement, and degree of nuclear crowding, which in some areas was so severe as to form bridges of epithelial cells extending across the glandular lumina. The cytoplasm was in general granular. Some apoptotic bodies, identified in all epithelial cell layers of the ducts and acini, were found in general in the intercellular space and occasionally seen in the cytoplasm of epithelial cells. The lumina always contained some macrophages, together with some apoptotic bodies. The frequency of mitoses in the epithelial cell layers was greater than in non-neoplastic prostate: PINlow, 0.053 (SEM 0.024)%; PINhigh, 0.095 (SEM 0.043)%. In both grades the percentage of mitoses was counted separately for the cells adjacent to the basement membrane, for the cells bordering the lumen, and for the cells in the position intermediate between the basal and the luminal layers. In both prostatic intraepithelial neoplasia grades the proportions of mitoses decreased from the basal position through the intermediate cell layers to the cell layer bordering the lumen. For PINlow, the mean values were 0.087 (SEM 0.04)% in the basal, 0.046 (SEM 0.033)% in the intermediate,

Figure 3 In contrast to the treated transition zone, there is inconspicuous branching of the ducts and acini within the peripheral zone of the non-neoplastic prostate. These look dilated and lined by atrophic epithelium.

Figure 4 Untreated prostatic intraepithelial neoplasia (low grade PIN). Cell crowding is present and accompanied by irregular nuclear spacing as well as an increase in nuclear size. Nuclei show finely granular chromatin and a few albeit prominent nucleoli usually located centrally.

and 0.024 (SEM 0.024)% in the luminal position. For PIN_{high}, the mean values were 0.194 (SEM 0.178)% in the basal position, 0.075 (SEM 0.06)% in the intermediate, and 0.049 (SEM 0.033)% in the luminal position. In PIN_{low} the percentages were slightly lower than in PIN_{high}.

In the treated patients prostatic intraepithelial neoplasia was observed only in the peripheral zone and was localised in scattered ducts in comparison with the control group in which the extension was greater. The epithelial cell lining showed a basal cell layer which was recognisable in most instances. A certain

degree of secretory cell type stratification was always present. However, crowding was less evident than in the untreated prostate because of cytoplasmic clearing and enlargement as a result of coalescence of vacuoles; the nuclei in general showed small inconspicuous nucleoli without the preferential margination typical of untreated prostatic intraepithelial neoplasia. The chromatin showed different degrees of changes which ranged from a mild condensation—which barely permitted the distinction between coarse chromatin granules (corresponding to heterochromatin) and finely dispersed chromatin (corresponding to euchromatin)—to a tightly condensed state very much like that observed in apoptosis. The luminal border of the epithelium was irregular in outline due to cells of different size and some shallow hollows corresponding to the site of detached cells. Apoptotic bodies were easily identifiable in all secretory cell type layers and were found only in the intercellular space. The lumina contained abundant cells: some were macrophages, some sloughed secretory cells with degenerative features, while others corresponded to apoptotic bodies. As the duct and acinar changes were so severe, no clear post-treatment grading of the prostatic intraepithelial neoplasia lesions was possible (fig 5). No mitotic figures were present in treated prostatic intraepithelial neoplasia.

PROSTATIC ADENOCARCINOMA

The hallmark of all the untreated adenocarcinomas was that the tumour nuclei were frequently multinucleolated, the nucleoli being prominent, marginated, and with a narrow clear space around. The chromatin in general looked finely granular with some chromatin margination along the inner surface of the nuclear membrane. The cytoplasm of the small and large acinar patterns was moderately clear, whereas in the cribriform and solid patterns it was generally granular. The cell boundaries were always clearly recognisable (fig 6). The closely packed glands and tumour nodules with cribriform or solid or trabecular patterns with amphophilic cytoplasm which looked haphazardly related to one another stood out on low magnification when compared with the pale-staining benign glands. Like the untreated prostatic intraepithelial neoplasia, some apoptotic bodies were identified in all epithelial cell layers and, together with macrophages, in the lumina.

When considering the mitotic count in the adenocarcinomas with a cribriform pattern, the proportions of mitoses, greater than in PIN_{high}, decreased from the basal position, or adjacent to the stroma, through the intermediate cell layers to the cell layer bordering the lumen. The mean values were 0.154 (SEM 0.096)% in the basal position, 0.072 (SEM 0.044)% in the intermediate, and 0.064 (SEM 0.04)% in the luminal position. In the solid or trabecular pattern the percentage of mitoses was calculated separately for the cells adjacent to the stroma and for the cells in the other cell layers. The mean value

Figure 5 Treated prostatic intraepithelial neoplasia (peripheral zone). There is inconspicuous branching of the ducts and acini, which look dilatated. The epithelial cell lining shows a recognisable basal cell layer. A certain degree of secretory cell type stratification is present. Crowding is less evident than in the untreated prostate. The lumina is rich in cells, including apoptotic bodies.

Figure 6 Untreated prostatic adenocarcinoma with an acinar pattern. Typical features are frequently multinucleolated tumour nuclei, prominent and marginalised nucleoli, surrounded by a narrow clear space. The chromatin looks finely granular with some chromatin marginalisation along the inner surface of the nuclear membrane.

in the cell layer adjacent to the stroma was 0.22 (SEM 0.111)% whereas in the other cell layers it was 0.074 (SEM 0.045)%. The proportions of mitoses were higher than for other categories of adenocarcinoma. The percentage of mitoses in the small acinar pattern was similar to that of PINlow and close to that in the large acinar pattern—that is, 0.058 (SEM 0.024)% and 0.068 (SEM 0.019)%, respectively. (The frequency of mitoses in the epithelial cell layers increased from benign prostatic hyperplasia through prostatic

intraepithelial neoplasia up to prostatic adenocarcinoma, the differences being significant for the following comparisons: Kruskal-Wallis test: benign prostatic hyperplasia *v* prostatic intraepithelial neoplasia *v* prostatic adenocarcinoma $p = 0.0001$; Mann-Whitney U test: benign prostatic hyperplasia *v* prostatic intraepithelial neoplasia $p = 0.006$, and benign prostatic hyperplasia *v* prostatic adenocarcinoma $p = 0.0003$. Prostatic intraepithelial neoplasia was regarded as a single category without distinguishing between low and high grade; prostatic adenocarcinoma was also regarded as a single category. Statistical analysis showed no significant differences for any of the comparisons between the different cell layers.)

In comparison with the untreated specimens, the treated tumours with acinar pattern showed neoplastic acini which looked shrunken and areas of individual infiltrating tumour cells separated by an abundant interglandular connective tissue. The epithelial tumour cells had inconspicuous nucleoli, nuclear shrinkage, chromatin condensation and pyknosis, cytoplasmic clearing and enlargement by coalescence of vacuoles and rupture of cell membranes (fig 7). Like the treated prostatic intraepithelial neoplasia, apoptotic bodies were easily identifiable in all epithelial cell layers and, together with macrophages and sloughed epithelial cells, in the lumina. The treated tumours with cribriform and solid or trabecular patterns showed nuclear and cytoplasmic changes induced by CET which looked less pronounced than in the acinar pattern. No mitotic figures were present in any of the treated adenocarcinomas.

Discussion

This study has shown that CET before radical prostatectomy causes regressive epithelial changes, together with enhanced apoptosis and reduced mitotic index on the non-neoplastic transition and peripheral prostate zones in prostatic intraepithelial neoplasia and prostatic adenocarcinoma.

Current medical opinion shows a lot of interest in alternatives to prostatectomy in the management of non-neoplastic prostate lesions called benign prostatic hyperplasia which affects the transition zone. In particular, antiandrogens such as LHRH agonists or flutamide have been used for reversing benign prostatic hyperplasia.^{18,19} Peters and Walsh demonstrated the possible effects of an LHRH agonist²⁰: the prostate regressed by about 24%, based on radiological imaging. A biopsy specimen of the prostate confirmed that the regression was primarily of glandular tissue, thus confirming earlier histological quantitative studies by Huggins and Stevens.²¹ This group of authors had already observed that various antiandrogen approaches decrease the epithelial component and produce histological atrophy of the prostate. Têtu *et al* reached a similar conclusion in a histological study investigating the effect of LHRH

Figure 7 Treated prostatic adenocarcinoma. In comparison with the untreated tissue, the treated adenocarcinoma with an acinar pattern shows neoplastic acini which look shrunken with areas of individual infiltrating tumour cells separated by abundant interglandular connective tissue. The epithelial tumour cells show inconspicuous nucleoli, nuclear shrinkage, chromatin condensation, and cytoplasmic clearing and enlargement.

agonist and flutamide.¹⁰ Others have drawn different conclusions about the possible clinical benefits and morphological changes of the medical castration approach. In a study by Keane *et al* biopsy specimens showed no evidence of reduction in epithelial cell height.²² Caine *et al* showed the clinical effectiveness of flutamide on benign prostatic hyperplasia.²³ However, no evidence of qualitative histological changes in prostatic biopsy specimens was observed. Our morphological findings in the transition zone, which basically are similar to those observed in prostatic intraepithelial neoplasia and prostatic adenocarcinoma, agree with the conclusions drawn by Huggins and Stevens,²¹ Peters and Walsh,²⁰ and Têtu *et al*.¹⁰

In the non-neoplastic peripheral zone, the main origin site of prostatic intraepithelial neoplasia and prostatic adenocarcinoma, we found diffuse changes which are different from those in the transition zone. This is a poorly recognised finding. To the best of our knowledge, Têtu *et al*¹⁰ were the only previous authors to mention very briefly that the glands at the periphery of the prostate treated with an LHRH agonist and flutamide were dilated and lined by atrophied epithelium. We do not have a definitive explanation for these morphological differences between the non-neoplastic prostate zones. Although the differential response might be linked to differences in hormone receptors, we advocate the alternative, traditional explanation for focal atrophy,²⁴—that is, as the consequence of ductal obstruction associated with a CET effect.

Prostatic intraepithelial neoplasia is considered to be a premalignant lesion which might progress to prostatic adenocarcinoma.^{13 14} Ellison *et al*⁸ and Têtu *et al*¹⁰ mentioned that CET has some kind of effect on prostatic intraepithelial neoplasia lesions. In particular,

the former group recently affirmed that the extent of high grade prostatic intraepithelial neoplasia decreased significantly in patients receiving a total androgen blockade. Even though the extent of prostatic intraepithelial neoplasia was evaluated visually, we reached the same conclusion in our study. In fact, prostatic intraepithelial neoplasia lesions were localised in scattered ducts compared with the control group in which the extension was greater. However, to our knowledge, the morphology of the regressing prostatic intraepithelial neoplasia lesions has not been described before. Our study showed regressive changes in the secretory cells, while the mitotic activity was abolished. The nuclei showed chromatin condensation and pyknosis. These changes are probably associated with qualitative and quantitative DNA alterations and might possibly be linked to apoptosis. A reduced DNA content was observed by Peters and Walsh²⁰ in non-neoplastic prostate. In their experience androgen deprivation for six months induced a 40% decrease in nuclear DNA content. This has also been our experience with flow cytometric analysis: in fact, DNA histograms with a hypodiploid mode were obtained from some of the fresh prostate specimens (unpublished data). The cytoplasmic changes are of the regressive type, in agreement with the study by Têtu *et al*,¹⁰ which showed that prostatic specific antigen immunostaining was weaker and the number of positive cells substantially reduced in prostatic adenocarcinoma treated by CET.

Apoptotic bodies were more frequently seen in the regressing prostatic intraepithelial neoplasia lesions than in the untreated lesions and were observed at the level of the cell layers as well as in the lumina. This was observed after CET in the normal and neoplastic prostate in studies in which it was suggested that CET induced a certain degree of epithelial regression by enhancing the cell death phenomenon called apoptosis.^{25 26} The findings of the present investigation with the experimental study of Szende *et al*²⁷ who investigated the effects of LHRH agonists and somatostatin on cancer in hamsters. They observed that the tumours treated with agonists showed regressive histological changes characteristic of apoptosis. Our observation probably corresponds to the finding of “progressive nuclear pyknosis” observed by Helpap²⁸ in patients receiving hormone treatment (the type of treatment was not specified). Apoptosis was evaluated by Stiens *et al*²⁹ in patients with prostatic carcinoma before and after oestrogen and radiation treatment. They observed that the apoptosis index can increase 10-fold within the first 10 days of treatment; under radiation treatment, apoptotic bodies can also be measured as increasing. Histologically, apoptotic bodies were seen in the intercellular space and in the lumen where some of the bodies appeared in the macrophage cytoplasm. This apoptotic body location supports the conclusions made by Kerr *et al*³⁰ on how apoptotic bodies are eliminated. This group reported that apoptotic bodies formed in

tissues are dispersed from their site of origin along intercellular spaces; some of those arising in the epithelia are extruded into the lumen, but they are also phagocytosed and degraded by the adjoining viable cells. Epithelial cells as well as cells of the mononuclear phagocytic system participate in this disposal. In malignant neoplasms many of the bodies are taken up and digested by surrounding neoplastic cells. Savill *et al* clearly demonstrated how macrophages present in the synovial cavity ingested apoptotic cells, mainly neutrophils, in acutely inflamed joints.³¹

The absence of mitoses in prostatic intraepithelial neoplasia lesions as well as in non-neoplastic prostate and prostatic adenocarcinoma indicates suppressed proliferation activity as a consequence of androgen deprivation treatment, as hypothesised by Murphy *et al*¹¹ and Bruchovsky *et al*.³² This has been documented in the neoplastic prostate.^{6 28 33-37} For instance, Zalutnai *et al* described the histological changes in rats with prostate cancer after treatment with somatostatin agonists and the D-Trp-6 analog of an LHRH agonist.³⁸ They reported that mitotic activity in the epithelium from the treated group was much lower than from the untreated specimens, in agreement with the findings obtained by Oomens *et al*,³⁴ who assessed the proliferative cell fraction of human prostatic carcinoma using Ki67. These authors found that the percentage of positive nuclei decreased significantly after androgen deprivation, dropping to 7% of the initial values after three months. Recently, Sakamoto *et al* found that short term treatment with LHRH agonists suppressed the activity of thymidine synthetase and thymidine kinase and substantially reduced the appearance of bromodeoxyuridine immunoreactive cells.³⁹ Armas *et al* reported decreased proliferating cell nuclear antigen (PCNA) expression due to preoperative androgen deprivation treatment on prostatic carcinoma.⁶ Magi Galluzzi *et al* also found that the values of the PCNA stained nuclei in treated cases were notably lower than in untreated specimens, and interpreted the low PCNA positivity not only as a decrease in proliferation but also as an attempt to repair DNA damage.⁹ Even though the proliferation activity is greatly affected, the stem cells might survive and form part of the morphological finding of basal cell prominence observed in the non-neoplastic transition zone and in prostatic intraepithelial neoplasia. In a study dealing with the effects of androgen withdrawal on the stem cell composition of Shionogi carcinoma, Bruchovsky *et al* experimentally documented the survival of this type of cells.³²

The morphological changes do not seem to be equally distributed over the prostatic adenocarcinoma lesions. Such unequal distribution seems to be linked to the tumour pattern. Those adenocarcinomas with an acinar pattern were greatly affected by CET: in fact, the architectural and cell damage is obvious and easily recognisable. CET exerted a pronounced effect, to the point that the tumour

acini shrunk, making it difficult to say whether they belonged to the large or small acinar pattern categories. In the cribriform and solid or trabecular pattern the changes were not so pronounced as in the acinar pattern, and some nuclear details were still recognisable, a feature also present in some ducts and acini with prostatic intraepithelial neoplasia. This means that the high grade lesions, whether infiltrating or intraductal, might give a poorer response to three months of CET, and that when high grade lesions are observed in a pre-treatment biopsy specimen, a longer period of CET has to be planned. Alternatively, as postulated by Akakura *et al*,⁴⁰ androgen suppression could be of the intermittent type. These authors studied the effects of intermittent androgen suppression on androgen dependent tumours and suggested that the replacement of androgens after a certain period of androgen suppression might result in the regeneration and differentiation of stem cells with further apoptotic potential. These cells could then be eliminated by successive androgen withdrawal.

In conclusion, CET induces regressive changes in the secretory cells and blocks the mitotic activity of the non-neoplastic prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma. In particular, its effect on prostatic intraepithelial neoplasia may be of paramount importance for the pharmacological treatment of prostate glands with a biopsy diagnosis of prostatic intraepithelial neoplasia without morphological or clinical evidence of prostatic adenocarcinoma. Further studies are necessary to investigate the longer term effect of CET and the importance of basal cell preservation in the regrowth of lesions when CET is discontinued.

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