Protein thiols in normal and neoplastic human uterine cervix

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Protein-thiol groups that react with dihydroxydinaphthyl disulphide during a 7 h incubation (so-called reactive protein thiols, PSH_r) have been quantitatively measured on sections of human uterine cervix by microcytospectrophotometry. Measurements were made on areas (1 μm^2) of epithelium and adjoining stroma in samples of normal cervix, and in samples obtained from patients with dysplasia, carcinoma-in-situ and invasive cancer. The ratio of PSH_r in epithelium to stroma is substantially reduced in the pathological conditions compared with normal and in apparently normal adjacent areas. Such changes in PSH_r are discussed in relation to the redox balance of the tissue, and free radical disturbances previously described.

Protein-thiol Cervix Cancer Microspectrophotometry Epithelium Stroma Field-effect

1. INTRODUCTION

Free radical disturbances are known to occur in many clinically important diseases, and in a variety of chemically induced tissue injuries [1–4]. Extensive and detailed studies on some experimental models of tissue damage have demonstrated clearly that the associated free radical disturbances can be of primary importance in the initiation of the gross metabolic and cellular perturbations that occur [5,6]. However, in many other situations where free radical disturbances have been proposed to contribute to pathological events, the evidence for the occurrence of reactive free radical intermediates and for their participation in the primary stages of tissue injury and disease is indirect, mainly as a consequence of the tissue concentrations of the presumed free radical intermediates being too low and too transient for direct detection by the definitive process of ESR spectroscopy [7]. The latter restriction does not apply to one type of human cancer that we have studied [8], carcinoma of the uterine cervix, where the difference in free radical content between normal healthy tissue and tumour material is substantial and readily detectable by ESR. A very strong ESR signal is detectable in normal samples of human cervix, whereas the signal is much reduced or absent in samples of the tumour [8].

The free radical species that is present in frozen powders of normal human cervix has features characteristic of a peroxy radical [9,10]. This type of free radical has oxidizing properties, and would have disturbing effects on the reduction-oxidation (redox) balance, including the ratio of thiol: disulphide groups (SH:S-S), especially around its locus of formation. Obviously, however, many other (and generally quantitatively more important) factors will also influence the thiol:disulphide balance in cells in situ.

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Because of the large differences in free radical content that occur between samples of normal and cancer tissue taken from human cervix, and the possibility that this difference will be associated with detectable perturbations of redox balance, we decided to investigate one aspect of the latter complex function of cellular metabolism using a quantitative microchemical method for protein-bound thiol groups. A review of this microspectrophotometric approach to these and related studies was recently presented to a symposium on cancer of the uterine cervix [11].

Previous studies have demonstrated relationships between the rates of cell division and the thiol contents of normal [12] and tumour cells [13,14]. However, those studies concentrated on the soluble thiol-containing substances of the cell, such as glutathione (GSH) and protein-thiols of the cytosol. Only a small amount of information is available from studies on cellular material on the variations in the content of thiol-groups that are not easily extractable with aqueous buffer or aqueous alcohol (e.g. protein-thiols firmly associated with biomembranes), although such thiol groups are probably of considerable importance in membrane function. A quantitative cytochemical method has been developed [15] to measure protein-thiol groups in fixed cells stained with 2,2-dihydroxy-6,6-dinaphthyl disulphide (DDD), and we have applied this procedure to our studies on sections of human uterine cervix.

Here we give results for the measurement of reactive protein-thiol groups (PSH_r) in epithelium and stroma of fixed sections of human uterine cervix, and demonstrate that the ratio of PSH_r in epithelium to that in stroma is considerably changed in sections prepared from samples taken from patients with dysplasia, carcinoma-in-situ or invasive carcinoma of the cervix compared with the normal situation. Moreover, measurements of PSH_r-values in apparently normal tissue adjacent to an area characteristic of the pathological disturbances just mentioned show trends similar to the changes observed in the lesions: these results are indicative of a diffuse change in redox balance that extends beyond the boundary of the lesion as defined by conventional histological evaluation. We believe these data are of considerable biochemical interest and may have potential clinical applications to pathological lesions of the uterine cervix, including carcinoma that, in some countries of the world, is a major cause of death by cancer in women [16].

2. MATERIALS AND METHODS

Human tissue samples were taken during operations for cone biopsy or hysterectomy. Normal samples of cervix were obtained mostly from patients undergoing hysterectomy for fibroleiomyoma of the uterine body with no evidence of significant pathological disturbances of the uterine cervix; none of the patients involved in this study had received medication additional to that required for surgery for one week prior to operation. None of the women involved in this report had been taking oral contraceptives in the period leading up to the operation.

Serial frozen sections of 10 μm thickness were prepared, using a TEK-11 cryostat, within 1 h of obtaining the samples at operation. The sections were fixed in methanol, and stained for reactive protein-thiol groups (PSH_r), by the methods of Nöhammer [15,17]. Alternate serial sections were stained with haematoxylin-eosin and evaluated histologically.

The extinctions of areas (1 mm^2) of epithelium and stroma of sections stained for reactive protein-thiol groups were measured using a Zeiss UMSP-1 microspectrophotometer with associated data processing and a rapid scanning table attachment. Full details of the procedures used, and of the microspectrophotometer sampling analysis can be found in reference [18]. Fast Blue B was obtained from Merck (Darmstadt); DDD was obtained from Sigma (Munchen).

3. RESULTS AND DISCUSSION

Table 1 shows data for reactive protein thiol groups in the epithelium and stroma of sections prepared from samples obtained from normal patients, patients with dysplasia, carcinoma-in-situ or invasive carcinoma. It can be seen that reactive-PSH groups are concentrated more in epithelium than in stroma; the ratio of reactive protein thiol groups in epithelium to stroma is significantly higher in sections prepared from normal patients compared to those prepared from patients with the

Table 1

Mean extinction values per unit area ($E/\mu m^2$) of sections prepared from samples of normal dysplastic and neoplastic human uterine cervix stained for reactive protein thiols

Group	N	Reactive protein thiols		
		Epithelium	Stroma	Epithelium / Stroma
1. Normal	53	0.34 ± 0.02	0.13 ± 0.01	2.73 ± 0.10
2. Dysplasia				
a. 'Normal tissue'	25	0.25 ± 0.03^{b}	0.14 ± 0.02^{ns}	1.81 ± 0.10^{e}
b. Dysplasia	33	0.27 ± 0.03^{ns}	0.18 ± 0.02^{c}	1.61 ± 0.09^{e}
3. Carcinoma-in-situ				
a. 'Normal tissue'	28	0.24 ± 0.04^{c}	0.11 ± 0.01^{ns}	2.10 ± 0.10^{e}
b. Carcinoma-in-situ	29	0.25 ± 0.03^{c}	0.16 ± 0.02^{a}	1.51 ± 0.06^{e}
4. Invasive cancer				
a. 'Normal tissue'	14	0.25 ± 0.04^{ns}	0.11 ± 0.01^{ns}	2.22 ± 0.20^{a}
b. Invasive cancer	29	0.22 ± 0.01^{c}	0.15 ± 0.01^{ns}	1.55 ± 0.08^{e}

Groups 2a, 3a and 4a give data obtained from apparently normal parts of the sections adjacent to the relevant lesions 2b, 3b and 4b. Mean values are given ± SE. Probabilities (P), obtained by Student's t-test, for the difference between the mean values in groups 2–4 vs the corresponding control value are shown as superscripts: ^{a} $P < 0.05$; ^{b} $P < 0.02$; ^{c} $P < 0.01$; ^{d} $P < 0.05$; ^{e} $P < 0.001$; ^{ns} not significant; N = number of individual cases

pathological disturbances studied.

The age range of patients in the normal groups was 29–81 years, with an average of 49 years. There was no discernible variation with age in the epithelium:stroma ratio in normal patients.

The number of samples of dysplasia studied is too small to enable detailed statistical analysis to be done concerning possible differences in the epithelium:stroma ratio in relation to grades of dysplasia; however, the data so far available suggest that such variations, if they occur at all, will be small in comparison to the differences between the normal and dysplasia groups (the corresponding mean values ± SE are 2.73 ± 0.10 vs 1.61 ± 0.09; table 1). Although no significant variations in the epithelium:stroma ratio have been detected between the various grades of dysplasia, there are progressive increases in the amounts of reactive protein thiols in both epithelium and stroma in the sequence: mild, moderate and severe dysplasia [19].

The ratio of reactive protein thiols in epithelium to stroma is decreased in lesion areas classified as dysplasia, carcinoma-in-situ or invasive cancer compared to normal (table 1). With dysplasia, the change in the ratio results largely from an increase in the reactive-protein thiol content of stroma with a small but not statistically significant decrease in the epithelium; with invasive cancer and carcinoma-in-situ the changes in the ratio are due both to a decreased reactive SH in the epithelium and a statistically significant increase in the stroma.

These differences in reactive protein-thiol groups of epithelium and stroma in lesions compared with normal tissue are consistent with a current concept that dysplasias and carcinoma-in-situ are different stages of the same disease-continuum (review [20]). However, it is clear from the results given in table 1 that there is no gradation of change in the ratio of the values found for reactive protein thiols in epithelium:stroma in moving from nor-

mal through dysplasias, carcinoma-in-situ to invasive cancer.

An important feature of the results found is that apparently normal epithelium and stroma (as evaluated by conventional histological criteria) show changes in the ratio of reactive-proton thiol groups in the epithelium:stroma that reflect at least in part those changes found in neighbouring regions of dysplasia, carcinoma-in-situ or invasive cancer. Indeed, in one instance a pathological lesion was well within the cervical canal whereas changes in reactive PSH_r groups in epithelium and stroma were easily observable in apparently normal regions around the external os. One possible interpretation for such a tendency is that the pathological lesions studied may exert a widespread effect on the reactive protein thiol content of neighbouring cells – such a field effect would have important biological implications and is under further investigation. However, we note that the changes found for reactive protein-thiol groups in areas of apparently normal tissue are not so marked as those found in the lesions and that also, the values obtained for areas of apparently normal tissue show rather greater variations between individual sections than observed for the corresponding lesion areas. Indications of a field effect in samples of human cervix obtained from patients with carcinoma-in-situ or invasive carcinoma have been reported by Millett et al. [21] who measured DNA content, and by Benedetto et al. [8] who studied the free radical content of cervix samples using ESR spectroscopy.

An alternative possibility to consider in evaluating the data presented here for apparently normal tissue surrounding an established lesion is that a rather generalised (i.e. diffuse) change has occurred in the area containing the morphologically identifiable lesion and pre-dating the lesion itself. Such a type of diffuse change has been referred to by Jensen et al. [22] in relation to angiogenesis induced by human breast tissue, who have suggested preneoplastic transformation is diffuse in samples obtained from patients with mammary cancer. Recent studies on virus infections of the cervix in relation to cancer of that tissue [23–25] may also be of relevance to the results described here: studies of the effects of herpes and papilloma virus infection on the reactive protein thiol content of human cervix are in progress.

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