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RECOGNITION OF CERVICAL NEOPLASIA BY THE ESTIMATION OF A FREE-RADICAL REACTION PRODUCT (OCTADECA-9,11-DIENOIC ACID) IN EXFOLIATED CELLS

S.K. TAY, A. SINGER, J.F.A. GRIFFIN,† D.G. WICKENS† and T.L. DORMANDY†

Department of Obstetrics and Gynaecology and †Department of Chemical Pathology, Whittington Hospital, Highgate Hill, London N19 5NF, U.K.

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The molar ratio between a diene-conjugated linoleic-acid isomer (18:2(9, 11)) and the parent linoleic acid (18:2(9, 12)), both esterified as phospholipids, was significantly different in exfoliated cells from normal cervices and from cervices with colposcopic and cytological evidence of precancer. The measurement may provide a simple and perhaps improved alternative to cytological screening.

KEY WORDS: Cervix, cancer, free radicals.

INTRODUCTION

Free radical activity in cell membranes results in the formation of conjugated dienes of polyunsaturated fatty acids. A single non-peroxide isomer of linoleic acid, 9-*cis*, 11-*trans* octadecadienoic acid (18:2 (9, 11)), accounts for more than 95% of the diene conjugates in human tissue.[1,2]

In a recent study the concentration of 18:2 (9, 11) and 18:2 (9, 12) were measured in normal, precancerous and cancerous uterine cervical biopsies.[3] The concentration of 18:2 (9, 11) and the molar ratio of 18:2 (9, 11) to 18:2 (9, 12) were significantly increased in cervical precancer and cancer. The concentration of 18:2 (9, 12) was not significantly different. This led to the study of 18:2 (9, 11) and 18:2 (9, 12) in exfoliated cells.

PATIENTS AND CONTROLS

The patients fell into two groups. Group 1 consisted of 39 women (mean age 29.7 years) randomly selected from new referrals to the Islington District Colposcopy Clinic, London. Group 2 were 21 women (mean age 31.2 years) who attended a hospital family planning and general gynaecological clinic for consultation unrelated to any cervical condition. All in Group 2 had had at least one normal cervical smear over the preceding six months and all gave permission for colposcopic examination and the taking of a further cervical smear.

Correspondence should be addressed to Dr T.L. Dormandy.

METHODS

Clinical

Colposcopy was performed in all women in both groups. After exposure of the cervix a smear was obtained with an Aires spatulum for routine Papanicolaou staining. Following the gentle removal of the remaining vaginal and cervical secretion with a cotton-wool swab a clean Aires spatulum was used to scrape the cervix three or four times. The scrapings were washed into 10 ml phosphate-buffer-saline (20 mmoles/litre Na_2HPO_4/KH_2PO_4, pH 8.0) containing potassium EDTA, 4.95 mmoles/litre. A colposcopically directed punch biopsy was taken from the transformation zone. This was subjected to histological examination and diagnosis according to the method and criteria described by Buckley *et al.*[4]

Biochemical

The method described for biopsy samples[3] was slightly modified (a) to improve biochemical stability and (b) to allow for the variable proportion of cells to extracellular material. The initial cell suspensions were centrifuged at 1600 g for 5 min. The supernatants were discarded: the cells were re-suspended in 1 ml NaCl, 154 mmoles/litre, containing $CaCl_2$, 40 mmoles/litre. 1 ml of a solution of Tris HCl buffer, pH 8.9, 0.1 mole/litre + methanol, 1 mole/litre containing Phospholipase A_2 (Sigma, Poole, Dorset, U.K.), 5000 I.U./litre was added. The suspension was incubated at 25°C for 15 min. 4 ml of a solution of acetic acid, 87 mmoles/litre, in methanol was added to precipitate the protein. After centrifugation the supernatant was applied to a Bond Elut column and eluted with 1 ml propan-2-ol: acetonitrile, 2:1. The eluate was dried under nitrogen and re-suspended in 50 μl of the same solvent. This was analysed by high-performance liquid chromatography as described by Iversen *et al.*[5]

RESULTS

The molar ratio of 18:2 (9, 11) to 18:2 (9, 12) was significantly higher ($p < 0.0001$) in the precancer group (Figure 1 and Table I). In addition, there was considerably less overlap between the two groups than observed in the previous study.

Despite the wide differences in the weight or cell count of the material available the 18:2 (9, 11)/18:2 (9, 12) molar ratio showed a remarkably narrow range (1.08–2.85) in normal subjects.

The 18:2 (9, 11)/18:2 (9, 12) molar ratio was highly significantly increased in the precancer group with an increased range (1.23–5.02).

DISCUSSION

Three considerations suggest that the measurement of 18:2 (9, 11) and 18:2 (9, 12) may provide a practical approach to the early detection of cervical cancer. First, in contrast to several other biochemical measurements which may become abnormal in cancer, the concentration of 18:2 (9, 11) is significantly increased in the earliest stage of precancer.

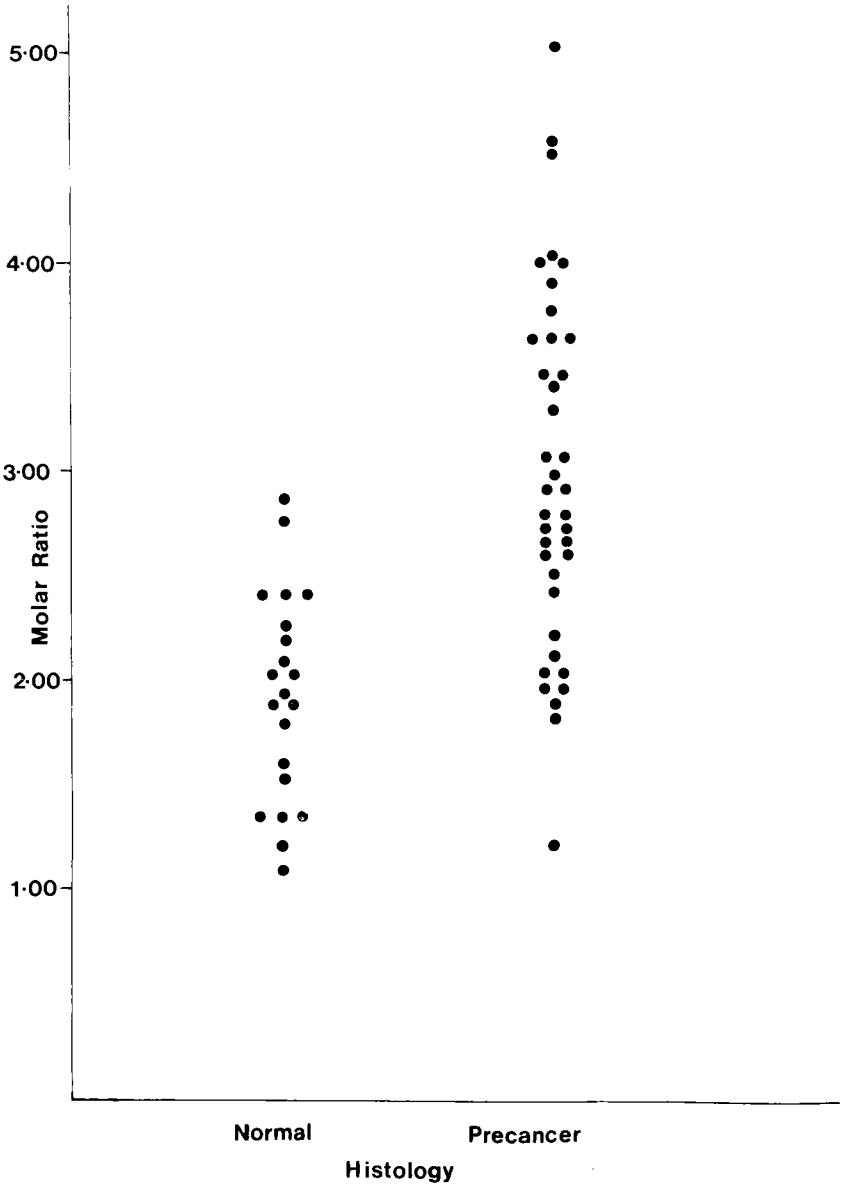

FIGURE 1 The 18:2 (9, 11) × 100/18:2 (9, 12) molar ratio in women with normal and abnormal histology.

Second, unlike most other biochemical discriminators the HPLC estimation of 18:2 (9, 11) incorporates its own reference measurement (18:2 (9, 12)).

Third, once established the HPLC method is quick, accurate, reproducible and non-observer-dependent.

TABLE I
The 18:2 (9, 11) × 100/18:2 (9, 12) molar ratio in cervical smears from normal women and women with CIN†

	n	$\bar{x}$	Median	S.E.	SD	Significance of difference t Test (v Normal)	Significance of difference Mann Whitney Test (v Normal)
NORMAL	21	1.91	1.92	0.11	0.50		
CIN 1	9	2.74	2.79	0.21	0.64	$p = 0.0045$	$p = 0.0030$
CIN 2	8	3.30	3.16	0.33	0.94	$p = 0.0042$	$p = 0.0008$
CIN 3	22	2.99	2.75	0.19	0.90	$p < 0.0001$	$p < 0.0001$
TOTAL CIN	39	3.00	2.90	0.14	0.85	$p < 0.0001$	$p < 0.0001$

† CIN = cervical intraepithelial neoplasia.

The increased free-radical activity in precancer and cancer reflected by the increase in the diene-conjugated linoleic-acid isomer, is in apparent contradiction to two recent developments in the study of the relationship between free radicals and neoplasia. First, neoplastic cells have been shown to be less susceptible to free-radical-mediated lipid peroxidation than are normal cells.[6] Second, Benedetto *et al.*[7] have found that the lipid peroxy free-radical electron-spin-resonance signal obtainable from the normal cervix becomes attenuated during cancerous change. Both these findings indicate diminished free-radical activity. The contradiction is apparent only because it depends on the widely held assumption that 'classical' peroxidation is the inevitable sequel of free-radical attack on polyunsaturated lipids in tissues.[8] The 18:2 (9, 11) isomer is not a peroxide: indeed, it contains no oxygen outside the terminal carboxyl group).[2]

The findings of this study suggest that the action of free radicals in neoplastic disease may follow a *non-peroxide* pathway, resulting in the generation of 18:2 (9, 11).

The results may have considerable practical relevance. The overlap between the normal and the precancer groups compares favourably with the rate of false negatives in cytological screening.[9,10] In two cases with abnormal molar ratios but negative cytology, colposcopy showed precancer which was confirmed by histology.

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