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Activities of Antioxidant Enzymes and Lipid Peroxidation in Endometrial Cancer

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Antioxidant enzyme activities and lipid peroxidation were analysed in normal endometrium and endometrial cancer tissues from Finnish and Japanese patients. The catalase and glutathione peroxidase activities of normal endometrium were significantly lower in Finns than in Japanese. Lipid peroxidation was slightly higher in endometrial cancer as compared with normal endometrium both in the Finns and in the Japanese. When cancer tissues were compared with normal endometrium both in Finns and Japanese the activity of superoxide dismutase was significantly lower in cancer tissue than in normal endometrium. In Finns glutathione S-transferase activity was also lower in endometrial cancer tissue than in normal endometrium, and a similar tendency was also found in Japanese. This study suggests that endometrial cancer tissue is associated with an impaired enzymic antioxidant defence system.
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INTRODUCTION

OBESITY IS one of the most important risk factors of endometrial cancer [1, 2]. This has usually been ascribed to the levels of peripheral aromatization of androgens to oestrogens in adipose tissue, which is the major source of oestrogens in postmenopausal women [3]. Several other dietary aspects may also be related to the risk of endometrial cancer. Total fat consumption *per capita* and incidence of endometrial cancer are positively correlated on an international scale [4]. Both the incidence of endometrial cancer and total fat consumption are low in Japan compared with Western countries. In a European case–control study [5] the risk of endometrial cancer was elevated in subjects reporting high fat intake. The basic mechanism by which dietary fats may modulate tumour development remains, however, largely unknown. One of the suggested mechanisms may be increased peroxidation and the production of active peroxides or their decreased inactivation [3, 6, 7].

In this study we have investigated antioxidant enzyme activi-

Table 1. Age and body mass index (BMI) in Finnish and Japanese endometrial carcinoma patients and in controls

	n	Age (years)	BMI
1	44	62.86 ± 12.82*	28.65 ± 5.90†
2	20	51.42 ± 9.25	25.58 ± 3.77
3	10	60.78 ± 8.33	23.56 ± 3.16
4	9	49.20 ± 7.06	23.37 ± 2.57

Age and BMI values are expressed as mean ±S.D. 1, Endometrial cancer (Finnish); 2, normal endometrium (Finnish); 3, endometrial cancer (Japanese); 4, normal endometrium (Japanese). * Different from Finnish controls ($P < 0.001$); † different from Finnish controls ($P < 0.05$).

ties and lipid peroxidation in endometrial cancer tissue of Finnish and Japanese patients and compared the results with those of normal endometrium as these nations show a large difference both in their fat consumption and in blood lipid profiles.

PATIENTS AND METHODS

Patients

The Finnish endometrial cancer patients were treated at the Department of Obstetrics and Gynecology of the University Hospital of Tampere. 41 of the patients had endometrial cancer stage I and 3 had stage II. 20 patients (controls) were treated for benign uterine disease, usually myomas or only metrorrhagia. The specimens from endometrial cancer tissue and from normal endometrium were taken after hysterectomy. None of the patients had radiotherapy, cytostatics or hormone therapy before surgery.

During the same time period corresponding specimens were taken from 10 Japanese endometrial cancer patients (stage I) and from 9 patients with uterine myomas treated at the Department of Obstetrics and Gynecology of Sapporo Medical College. The mean age and mean body mass index [8] of the patients are shown in Table 1. Cancer patients are older than the controls as it is difficult to receive enough tissue from the atrophic endometrium of postmenopausal women. All biochemical analyses were carried out in one laboratory in Finland. The samples were shipped from Japan to Finland in dry ice, and all samples were stored at −70°C until analysed.

Chemicals

Butylated hydroxyanisole, cumene hydroperoxide, epinephrine, glucose-6-phosphate, hydrogen peroxide, reduced glutathione (GSH), reduced nicotinamide dinucleotide diphosphate (NADPH), as well as the enzymes catalase (bovine liver), glutathione reductase (baker's yeast) and superoxide dismutase (Cu/Zn-form, bovine erythrocytes) were all purchased from Sigma Chemicals Co.

Tissue preparation

Tissue samples were rinsed in saline and weighed. A 20% (w/v) homogenate was prepared in a 0.25 mol/l sucrose solution (0°C) with a Potter–Elvehjem glass–teflon homogeniser driven by an electric drill at 500 rpm. A postmitochondrial supernatant was prepared by centrifugation (10 000 *g* max for 10 min at 4°C).

Lipid peroxidation

The appearance of conjugated diene double bonds in polyunsatured fat was used to estimate the level of oxidative stress in tests. Lipids were extracted from tissue homogenates by chloroform–methanol, dried under nitrogen atmosphere and then redissolved in cyclohexane, were analysed spectrophotometrically (at 232 nm) for quantitation of the diene conjugation [9]. Thiobarbituric acid reactive material (mainly malondialdehyde) was measured according to [10]. As sucrose solution interferes with thiobarbituric acid reactive materials as far as absorbance wavelengths are concerned, an adequate sucrose blank was used.

Enzyme assays

All enzyme activity measurements were done under optimal conditions with respect to incubation time and protein concentration. Superoxide dismutase (Cu/Zn-form) was assayed spectrophotometrically by inhibition of epinephrine auto-oxidation [11]. Catalase activity was determined by measuring the rate of disappearance of 15 mmol/l hydrogen peroxide at 240 nm [12]. The activities of glutathione peroxidase (with cumene hydroperoxide as the substrate) [13], glutathione transferase (with 1-chloro-2,4-dinitrobenzene as the substrate) [14] and hexose monophosphate shunt [15] were measured spectrophotometrically by the methods described. The apparent activities of superoxide dismutase and catalase activities determined in tissue homogenates whereas the 10 000 *g* supernatant was used in glutathione peroxidase, glutathione S-transferase and the hexose monophosphate shunt assays.

Proteins

Protein content was measured by the Biuret method [16] with bovine serum albumin as the reference protein.

Statistical methods

Statistical analysis of the data employed a one-way analysis of variance followed by *t*-test. Statpack Gold Statistical Analysis Package (Walonic Associates, Inc.) was used in the analyses.

RESULTS

Lipid peroxidation was measured by the amount of thiobarbituric acid reactive material and diene conjugation. No significant differences were found in these parameters between Finnish and Japanese endometrial cancer patients or between cancer patients and controls although in both countries cancerous patients had slightly higher lipid peroxidation levels (Table 2).

When cancer tissues were compared with normal endometrium both in Finns and Japanese, the activity of superoxide dismutase was found to be significantly lower in cancerous than in normal endometrium (Table 3). Glutathione transferase activity was lower in endometrial cancer tissue than in normal endometrium. In normal endometrium catalase and glutathione peroxidase activities were lower in Finns than in Japanese

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Table 2. Lipid peroxidation expressed as thiobarbituric acid reactive material (TBA) (nmol/g fresh tissue) and conjugated dienes (ABS/g fresh tissue) in Finnish and Japanese endometrium cancer patients and in controls

	TBA	Conjugated dienes
1	41.2 ± 30.5	1.90 ± 0.95
2	33.6 ± 25.5	1.27 ± 0.68
3	40.7 ± 14.6	2.87 ± 1.31
4	34.4 ± 8.7	2.09 ± 0.30

Values are expressed as mean ±S.D. 1, Endometrial cancer (Finnish); 2, normal endometrium (Finnish); 3, endometrial cancer (Japanese); 4, normal endometrium (Japanese).

(Table 3). However, there was insufficient tissue for catalase measurements in the Finnish patients.

There were no statistical differences in the amounts of diene conjugation, thiobarbituric acid reactive material or antioxidant enzyme activities between patients with a body mass index > 30 and the others (data not shown).

DISCUSSION

Endometrial cancer is a hormone-dependent tumour and postmenopausally the peripheral conversion of oestrogens has been considered an important aetiological factor [1, 2]. Increased fat intake increases oestrogen synthesis which is related to enhanced conversion of androgens to oestrogens in adipose tissue [3]. In this study obesity was a common finding among endometrial cancer patients. High intake of dietary fat has been suggested to result in increased formation of lipid peroxides and in some tissues lipid peroxidation reactions and an impairment in the antioxidant defence system have been suggested to be related to carcinogenesis in humans and tumour promotion in rats [3, 6, 7]. In the Finnish population, which has a high dietary fat intake, normal endometrial activities of superoxide dismutase, catalase and glutathione peroxidase were significantly lower than in Japanese control subjects. This suggests that the amount of dietary fat might modify the enzymic antioxidant defence system. It is not well understood how membrane lipid turnover, lipid peroxidation and reactive oxygen species interact with cellular antioxidant capacity. However, earlier studies have suggested that lipid peroxidation reactions also, e.g. following single exposure to UV irradiation [17, 18], are accompanied by decreased superoxide dismutase activity.

Oestrogens are capable of interfering with lipid metabolism as they, e.g. increase serum phospholipid levels [19, 20], and steroid hormones may also have an effect on the level of oxidative stress as oestrogens have been suggested to act as inhibitors of lipid peroxidation [21]. Earlier studies on the role of lipid peroxidation in carcinogenesis are conflicting as the results vary from one tissue to another. Elevated levels of lipid peroxidation products have been reported, e.g. for liver cancer [22], for colorectal cancer [23] and for human breast cancer [24], while either unchanged or decreased levels of lipid peroxidation have been reported to be associated with induced breast cancer [25], rat hepatoma [26, 27] and mouse skin exposed to a tumour promoter [28]. Experimental studies on rats fed various fat diets suggest that enhanced oxidative stress may be related to the development of chemically induced cancers [6, 7].

To our knowledge there is no previous information on the role of lipid peroxidation and the enzymic antioxidant defence system in endometrial cancer. Our results demonstrated lower superoxide dismutase levels in endometrial cancer than in normal endometrium both in the Finns and Japanese. Changes in superoxide dismutase activity can be considered to be of biological significance since superoxide dismutase, which acts by trapping superoxide and thereby defends cells against peroxidation reactions, is one of the most important antioxidant enzymes. Glutathione transferase activity was also lower in cancer than in normal endometrium in the Finns, and in normal endometrium both catalase and glutathione peroxidase activities were lower in Finns than in Japanese.

Recent studies have suggested that point mutations of *ras* oncogenes represent a relatively frequent genetic alteration in human endometrial cancer [28]. *Ras* oncogenes induce changes in membrane lipid turnover [29,30] and, therefore, it is possible that the observed changes in the activities of the antioxidant enzymes could be related to the effects of the *ras* oncogene on membrane lipid turnover. The role of lipid peroxides and antioxidant enzymes in carcinogenesis is not completely understood, and changes in lipid peroxidation reactions and in antioxidant defence systems have been reported to be associated with changes in a variety of biochemical pathways. There is some evidence suggesting that the level of oxidative stress is involved

Table 3. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GT) and hexose monophosphate shunt (HMS) activities in Finnish and Japanese endometrial cancer patients and in controls

	SOD (μg/mg protein)	CAT (μg/mg protein)	GPX (nmol/min/mg protein)	GT (nmol/min/mg protein)	HMS (pmol/min/mg protein)
1	1.67 ± 1.67*	N.D.	2.63 ± 1.65	3.43 ± 2.16‖	309.33 ± 230.33
2	2.82 ± 1.98	5.96 ± 2.24‡	1.90 ± 0.76§	7.22 ± 4.80	426.13 ± 287.42
3	1.86 ± 1.00†	10.00 ± 5.07	3.78 ±2.14	4.88 ± 4.46	177.22 ± 284.78
4	4.04 ± 2.93	12.82 ± 5.08	3.70 ± 1.06	6.48 ± 2.04	222.40 ± 265.47

1, Endometrial cancer (Finnish); 2, normal endometrium (Finnish); 3, endometrial cancer (Japanese); 4, normal endometrium (Japanese).

* Different from normal endometrium (Finnish) ($P < 0.05$); † different from normal endometrium (Japanese) ($P < 0.05$); ‡ different from normal endometrium (Japanese) ($P < 0.001$); § different from normal endometrium (Japanese) ($P < 0.05$); ‖ different from normal endometrium (Finnish) ($P < 0.001$).

in carcinogenesis by regulating, e.g. the expression of oncogenes [31] and recent studies have also suggested that reactive oxygen species are capable of regulating protein kinase C activity [32] which, in turn, is apparently involved in regulation of cellular differentiation and proliferation [33].

The present study indicates that endometrial cancer tissues have an impaired enzymic antioxidant defence system. Previously, e.g. decreased superoxide dismutase activity has been reported in various hyperproliferative keratinocytes including squamous cell carcinoma and benign hyperproliferative skin such as psoriatic epidermis [34, 35]. On the basis of the current knowledge we cannot exclude the possibility of altered antioxidant defence system and lipid peroxidation reactions being a secondary phenomenon related for instance to changes in membrane lipid turnover or cell proliferation.

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