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Letters

In vivo Drug Intracellular Localisation by Analytical Ion Microscopy: Preliminary Study in Gastric Adenocarcinomas Treated with 5-fluorouracil

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THE PRECISE antitumour activity of 5-fluorouracil (5-FU) remains uncertain but at least two mechanisms of action are involved causing cell injury: inhibition of thymidylate synthetase and incorporation into RNA [1]. Although the penetration of this drug into the cells of neoplastic tissue is critical for this activity, the *in vivo* distribution between normal and tumour cells is unknown. Recently, analytical ion microscopy (AIM), the first available method capable of mapping chemical elements in tissue sections [2, 3], has been employed to detect the fluorine (F) content of 5 fluor-2′deoxyuridine in cultured cells [4]. The aim of this preliminary study was to localise and quantify the fluorine of 5-FU found in cell nuclei of human biopsies.

11 patients with gastric adenocarcinomas were included. Biopsies of gastric mucosa were obtained during endoscopy before the initiation of treatment (6 patients, group I), 15 min after the beginning of chemotherapy with 5-FU at a dose of 1 g/m^2/day (4 patients with oral consent, group II) and 23 days after 5-FU perfusion (2 patients, group III). Fragments first chemically fixed were embedded in methacrylate resin. Semithin sections (1 micron in thickness) were used as histological controls and serial semithin sections (3 microns) were deposited on ultrapure gold holders for ion analysis. Mass resolution (M/ΔM 2000) was used in order to eliminate interferences between cluster ions and specific ions studied. The elemental analytical images were displayed on a fluorescent screen connected to an image analysis system [5]. The fluorine beam intensity was also measured, using an electron multiplier, on nuclei selected with a special aperture (1.5 micron).

The main advantage of AIM is its ability to map fluorine (Fig. 1, lower) in relation to the histological structure which can be observed simultaneously on the optical serial semithin section (Fig. 1, upper). Fluorine was never observed in patients in group I and III and only detected in group II patients who had received a 5-FU perfusion. It was mainly observed in the nuclei of normal and neoplastic gastric cells. Its concentration, measured in 30 nuclei per section, ranged from 1.7 to 3.10^{-4} arbitrary units.

This study demonstrates for the first time the localisation of a cytotoxic drug (5-FU) in human tissue without the need of radioactive labelling. Numerous mechanisms of chemoresistance of tumour cells have been evoked; one of them might be the absence of drug penetration. The quantification of fluorine using the AIM will permit the comparison of normal and tumour cell uptake. We are now investigating the interest in fluorine mapping with regard to clinical response to treatment.

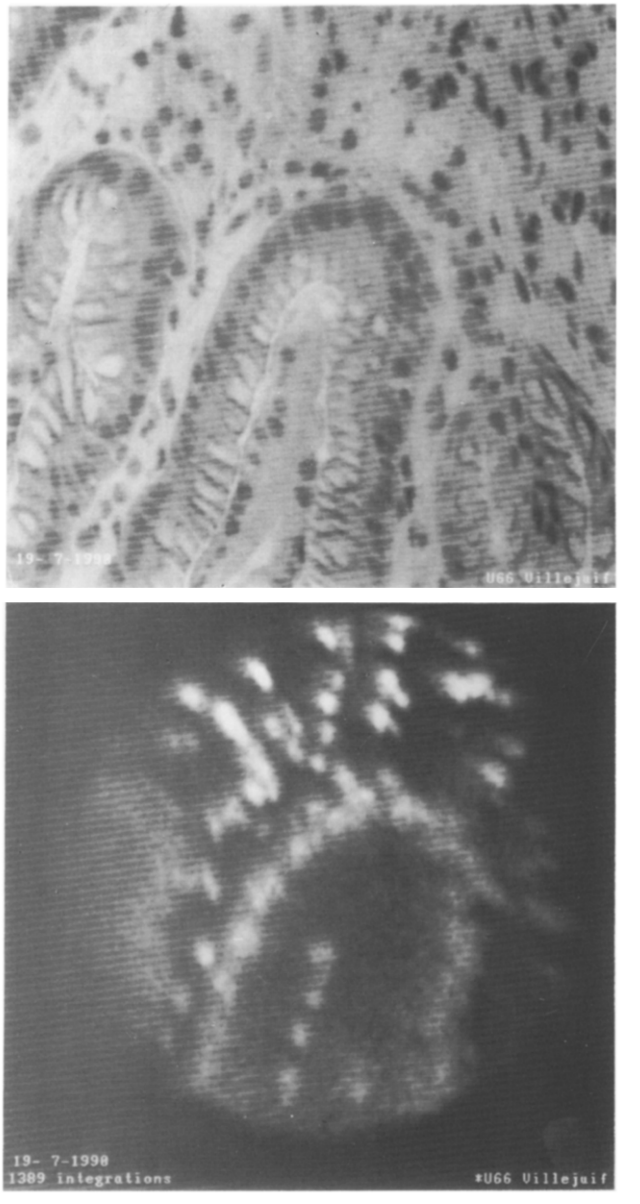

Fig. 1. AIM mapping of fluorine (lower) into cell nuclei located on optical serial section (upper). Human gastric mucosa was obtained by biopsy during treatment with 5-FU for gastric adenocarcinoma.

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Vitamin A, Gonadotropins and Ovarian Cancer

P.K. Heinonen

THE AETIOLOGY of ovarian cancer is still obscure. Ovarian cancer is common in countries, e.g. those of Scandinavia, with high consumption of milk products [1]. Cramer and colleagues reported that lactose consumption may be a dietary risk factor of the ovarian cancer [2]. This is linked to galactose consumption, and galactose metabolism with hypergonadotropic hypogonadism. Other dietary factors may affect gonadotropins. Vitamin A (retinol) is essential for reproductive function (oogenesis) in the female. It is also an important element in differentiation and proliferation of epithelial tissues [3]. Retinol cannot synthesised in the body and must therefore be taken in with the food. Milk is one natural source of fat-soluble vitamin A in the diet.

I have studied serum levels of retinol and gonadotropins in postmenopausal women with epithelial ovarian tumours. Serum was obtained from 52 women (mean age 64 years, range 48–83). 16 of them had epithelial ovarian cancer, 16 benign ovarian tumours and 20 were healthy controls without ovarian neoplasms. The samples were collected before any therapeutic intervention. Serum follicle-stimulating hormone (FSH) and luteinising hormone (LH) were measured by radioimmunoassay methods [4]. Serum retinol was measured by high performance liquid chromatography and ultraviolet detection [5]. Correlations were derived using a computer program for linear regression.

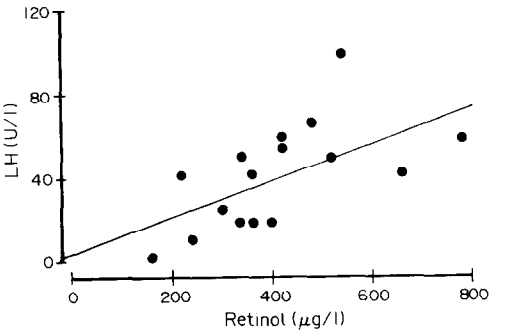

Fig. 1. Correlation between serum luteinising hormone (LH) and retinol in postmenopausal women with ovarian cancer.

A significant positive correlation ($r = 0.61$; $P = 0.012$; Fig. 1) was noted between serum LH and retinol concentrations in women with ovarian cancer, but not in patients with benign ovarian tumours ($r = 0.04$; $P = 0.88$) nor in control subjects ($r = 0.09$; $P = 0.72$). Serum concentrations of retinol and FSH did not correlate significantly in women with ovarian cancer ($r = 0.21$; $P = 0.94$), with benign ovarian tumours ($r = 0.09$; $P = 0.73$) nor in control subjects ($r = 0.229$; $P = 0.22$). The mean serum levels of retinol and gonadotropins were similar in three groups studied.

The relationship between retinol and LH has not been reported previously in women with ovarian cancer. Retinol may stimulate LH secretion or have LH-like effect on ovarian tissue. Retinol stimulates steroidogenesis *in vitro*, although the mechanism is not known [6]. According to one theory, increased stimulation of gonadotropins may directly or via steroid hormones affect differentiation, proliferation and malignant transformation of the surface epithelium of the ovary [7].

Retinol and its derivatives have, in general, anticarcinogenic potential in many epithelial tissues [3]. According to the present preliminary finding, retinol may have rather the opposite role in the pathogenesis of epithelial ovarian cancer.

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