Pergamon

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Short Communication

Retinoic Acid Concentrations in Patients With Squamous Cell Carcinoma of the Head and Neck

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The serum concentrations of all-trans (atRA) and 13-cis (13cRA) retinoic acid were determined by high performance liquid chromatography in 27 patients with squamous cell carcinoma of the head and neck and in 80 healthy controls. This investigation seemed relevant as ethanol is an aetiological factor in these cancers and has been suggested to interfere with the synthesis of atRA. Neither the serum concentration of atRA nor that of 13cRA differed between patients and controls. The serum atRA concentration did not differ between fasting and non-fasting patients, but the serum 13cRA concentration was significantly higher in non-fasting than in fasting patients, probably due to the dietary retinoid content.

Key words: retinoic acid, squamous cell carcinoma, head and neck cancer
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INTRODUCTION

VITAMIN A IS essential for normal growth and differentiation of epithelial cells, including those lining the oral cavity and upper aerodigestive tract. Squamous cell metaplasia of respiratory tract epithelia is an early sign of vitamin A deficiency. Retinoid deficiency is associated with an increased incidence of cancer, and retinoid supplementation may inhibit malignant transformation. In this context, the effects of retinoids are mediated at least via all-trans and 9-cis retinoic acid, two highly potent metabolites of retinol. As normal diet contains retinol but very little retinoic acid, the retinoic acids, all-trans, 9-cis and 13-cis, are produced in the body by oxidation of retinol [1].

As ethanol is an aetiological factor in these cancers [2] and has been suggested to interfere with the synthesis of all-trans retinoic acid [3], the aim of the present study was to determine the serum concentrations of all-trans and 13-cis retinoic acid in patients with untreated squamous cell carcinoma of the oral cavity, pharynx or larynx.

PATIENTS AND METHODS

Venous blood was obtained from 27 patients, seven women and 20 men, aged 31–81 years (mean 64 years). All patients had an untreated squamous cell carcinoma, verified by histopathological examination. The distribution of tumour localisation and stage is listed in Table 1. Of the 27 patients, 16 were smokers and seven were alcohol-abusers. None of the patients were treated with anticonvulsants, which have been shown to lower the serum retinoic acid concentration [4]. Serum was recovered by centrifugation and stored at −20°C in the dark until analysis, care being taken to avoid any exposure to light. In 14 patients, fasting (>8 h) blood samples were obtained. The control group comprised 80 non-fasting blood donors, 40 men and 40 women, 20–60 years old.

All-trans and 13-cis retinoic acid concentrations were measured with high performance liquid chromatography (HPLC) as described by Wyss and Bücheli [5]. The calibration standards—pure all-trans and 13-cis retinoic acid and the internal standard Acitretin—were gifts from Hoffmann-La Roche. The retinoids (weighed to concentration) were dissolved in ethanol, and aliquots added to a charcoal-treated serum matrix which was stored in the dark at −70°C. All handling of samples

Table 1. Tumours listed according to localisation and stage (I–IV)

Tumour localisation	Stage I	II	III	IV
Oral cavity	0	1	3	9
Oropharynx	0	3	1	6
Hypopharynx	0	0	0	1
Larynx	1	1	0	1

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Table 2. Serum concentrations (nmol/l) of all-trans and 13-cis retinoic acid (means ± S.D.) in patients with squamous cell carcinoma of the head and neck and healthy blood donors

Category	all-trans retinoic acid	13-cis retinoic acid
Patients ($n = 27$)	5.0 ± 1.1	4.4 ± 1.6
Blood donors ($n = 80$)	5.2 ± 1.0	4.8 ± 1.3

and retinoids was done in the dark or under yellow light. The coefficient of variation (CV%) for HPLC was 5.5% for all-trans retinoic acid and 5.9% for 13-cis retinoid acid (both at a level of ≈5 nmol/l). Normality of distribution was tested with the Shapiro–Wilk test, and group differences with Student's *t*-test.

RESULTS

The serum concentrations of all-trans and 13-cis retinoic acid did not differ between patients and controls (Table 2). The serum concentrations of 13-cis retinoic acid were significantly higher in non-fasting than in fasting samples [5.3 ± 1.5 nmol/l ($n = 13$) versus 3.5 ± 1.3 nmol/l ($n = 14$), $P < 0.001$], probably due to the dietary content of retinoids, whereas those of all-trans retinoic acid did not differ between fasting and non-fasting samples. This is in accordance with the findings of Eckhoff and coworkers who reported that the increase in 13-cis retinoic acid after an oral dose of retinyl palmitate was greater than that of all-trans retinoic acid [6]. Despite the aforementioned difference in serum concentration of 13-cis retinoic acid between fasting and non-fasting samples, and the lack of corresponding difference in all-trans retinoic acid concentrations, there was significant correlation between the 13-cis and all-trans retinoic acid concentrations, both in fasting samples [$r = 0.567$ ($n = 14$), $P < 0.05$] and in non-fasting samples [$r = 0.396$ ($n = 93$), $P < 0.001$]. However, there was no correlation between the serum concentrations of either retinoic acid and patient age, tumour stage or tumour site.

DISCUSSION

The physiological importance and regulation of the concentrations of the retinoic acids in plasma and tissues is poorly understood. Nor has any function yet been attributed to 13-cis retinoic acid as it does not bind to retinoic acid receptors (RARs). Many tissue cells can convert retinol to retinoic acid, and those unable to do so may derive retinoic acid from the calculation. The concentrations of all-trans retinoic acid and 13-cis retinoic acid found in this study are in good agreement with those previously reported for healthy volunteers [7–8].

All-trans retinoic acid is catabolised by enzymes belonging to the P-450 family. Conceivably, genetically determined rapid catabolism of all-trans retinoic acid might explain an increased cancer risk in smokers. Moreover, alcohol has been suggested to inhibit the synthesis of all-trans retinoic from retinol [3], although we have consistently found 'normal' all-trans and 13-cis retinoic acid levels in alcohol-abusing males (data not shown). Like our patients, many patients with squamous cell carcinomas of the upper aerodigestive tract use tobacco and consume alcohol. In this study, we did not find the serum concentration of all-trans retinoic acid to be reduced in head and neck carcinoma patients. This does not exclude the possibility that there exist subgroups of head and neck cancer patients with abnormal catabolic rates of retinoic acid [9].

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